Advanced engineering of Fibrous Non-steroidal anti-inflammatory drug (NSAID) films for buccal application using Electrohydrodynamic Atomisation

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Abstract

The development of therapeutic dosage (e.g. pharmaceutical) systems is an ongoing process which, in recent times has incorporated several emerging disciplines and themes at timely intervals. While the concepts surrounding dosages have developed and evolved, many polymeric excipients remain as the preferred choice of materials over existing counterparts, serving functions as matrix materials, coatings and providing other specific functional properties (e.g. adhesion, controlled release and mechanical properties). Therefore, polymer is employed as a matrix carrier of materials or as active release performance modulating agents for polymeric based dosages.

There have been, however, developments in the deployment of synthetic polymeric materials (e.g. polycaprolactone, poly lactic co-glycolic acid) when compared to naturally occurring materials (e.g. lactose, gelatin). Additionally, numerous techniques have been advanced further to novel engineering polymeric structures which provide materials in micrometer to nanometer scale range.

Some of these structures enabling technologies include spray drying, super critical processing, microfluidics and even wet chemical methods. Recently maturing processes which is operational at the ambient environment is electrohydrodynamic (EHDA) engineering methods (ES and ESy). They have emerged as robust technologies offering potential to fabricate a plethora of generic structures directly into a fibrous polymer matrix system (e.g. particles, fibres, bubbles and pre-determined patterns) on a broad scale range.

This research focuses on key developments using EHDA technology for the pharmaceutical and biomaterial remits when selecting synthetic and/or naturally occurring polymers as pharmaceutical (and therapeutic) excipients. EHDA was employed to engineer NSAID drugs in fibre film form for buccal delivery. EHDA was selected to develop fibre film of Indomethacin, Diclofenac Sodium, Ketoprofen, and Piroxicam in cooperation of PVP, Ethocel, Methocel, HPMC, and Tween 80.

Morphology of electrospun films were analyzed by SEM and further characterized using DSC, TGA, FTIR, Raman and XRD. DSC and XRD demonstrated NSAID drugs change from crystalline to amorphous state. FTIR and Raman data suggest NSAID, PVP and co-polymers (Methocel™ E5, Methocel™ E15 Ethocel™ E10, HMPC and Tween® 80) were integrated in stable fashion into filamentous structures via ES.

The release behaviour from several matrixes that was observed suggesting a potential route to modify drug release based on polymeric excipients. Therefore, identifying co-polymers in matrix and their effect in vitro release was the main core of chapter 3. Based on the results (fast or slow release), the co-polymer was incorporated with several NSAID drugs. However, each NSAID drugs show different release behaviour with same co-polymer.

In addition, the underlying EHDA process principles are discussed along with key parameters and variables (both materials and engineering). EHDA technologies are operational at ambient conditions and recent developments have also demonstrated their viability for large scale production. These are promising technologies which have potential in established (e.g. films, dressings and microparticles) and emerging scientific themes (e.g. nanomedicines and tissue engineering). Moreover, EHDA a one-step process facilitates us to optimise dosage forms as desirable in a single step for several age groups.

The use of EHDA need to be more explored within the buccal research concern. It has shown great potential in this research concept; therefore, it is viable to pursue to wider area in this field.

Declaration

The work described in this thesis was performed by the author in the School of Pharmacy within the Faculty of Health and Life Sciences (De Montfort University). I declare all the work presented in this thesis has been undertaken by myself.

The work was performed by the author and has not submitted in any other form for any other degree or qualification.

Signed:

Date:

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Publication and conferences

Publication

Haj-Ahmad, R. et al. (2015) EHDA Spraying: A Multi-Material Nano-Engineering Route. Current Pharmaceutical Design 21, pp.3239-3247.

Rsekh, M. et al (2016). Polymeric Based Therapeutic Delivery Systems Prepared Using Electrohydrodynamic Processes. Current Pharmaceutical Design, 22, pp, 2873-2885.

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Conferences

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Table of Contents

List of Equations

List of Figures

List of Tables

Abbreviations

API	Active Pharmaceutical Ingradient
NSAID	Non- Steroidal Anti-inflammatory drugs
PAT	Process analytical technology
AV	Applied voltage
EHDA	Electrohydrodynamic Atomisation
CoEHDA	Coaxial Electrohydrodynamic Atomisation
ESy	Electrospraying
COES	Coaxial Electrospraying
ES	Electrospinning
FR	Flow rate
ST	Surface tension
COEP	Coaxial Electrospinning
DD	Drug delivery
DSC	Differentia Scanning Calorimetry
TGA	Thermal Gravimetric Analysis
FTIR	Fourier Transform Infrared Spectroscopy
RS	Raman Spectroscopy
SEM	Scanning Electron Microscope
AFM	Atomic force microscopy
XRD	X-ray diffraction
EE	Encapsulation efficiency
ICH	International Conference on Harmonization
QBD	Quality by design
CQAs	critical quality attributes
DoE	Design of Experiment
QTPP	Quality Target Product Profile
CMAs	Critical Material Attributes
CPPs	Critical Process Parameters
PID	proportional, integral and Derivative

RA	Risk Assessment
µl	Microliter
min	Minutes
nm	Manometer
µm	Micrometre
NP	Nano particle
NF	Nano fibre
MPs	Micro particles
PVP	Polyvinylpyrrolidone
Etho	Ethocel
Metho	Methocel
HPMC	Hydroxypropyl Methacrylate
T80	Tween 80
Indo	Indomethacin
Keto	Ketoprofen
Pirox	Piroxicam
Diclo	Diclofenac Sodium
W/W	Weight per Weight
W/V	Weight per Volume
W/O	Water in Oil
O/W	Oil in Water

Chapter 1 introduction

1.1 Background

The most important discipline of pharmacy is pharmaceutics which is related to the science of pharmaceutical formulation and drug delivery. Pharmaceutic scientists prepare an active pharmaceutical ingredient into a medication for patients use in a safe and effective manner (Platt et al., 2008). Over the past decades, considerable advances have been made through the science of drug delivery system and technology to pass the drug into the body (Zhang et al., 2017; Yi and Kosel, 2017). Therefore, despite notable advances in the drug delivery system development, formulation and therapeutic agent and targeting to specific part of the body remain one of the most researched aspect within pharmaceutics. Lack of efficient drug at the site of action and body elimination of drug are the major drawbacks for drug delivery systems (Kiryukhin, 2014). During last decades there have been noteworthy growth in controlling the drug release of an active drug which is the key for drug efficacy. Drug targeting with specific drug delivery systems methods help to achieve optimal concentration range and as a result overcome toxicity of therapeutic and defence mechanisms of host. The development of targeting delivery (drug active in the target area) and releasing drug within period (sustain release) are the current efforts for drug delivery scientists. Moreover, many developments have been achieved to control the concept of pharmacokinetics, non-specific toxicity, pharmacodynamics, immunogenicity, bio-recognition and effectiveness of the delivery system. Therefore, understanding the combination of many factors such as polymer science and chemistry, biological system knowledge and pharmaceutics play part to develop the ideal delivery system (Mishra, Nayak and Dey, 2016; Debele, Mekuria and Tsai, 2016; Chahibi, 2017).

Parental and oral routes of administration were traditional choice as liquid or tablets; however, they also had many drawbacks subordinated with them. By utilising alternative routes of administration can overcome some of disadvantages (Gurruchaga et al., 2015). Medicines are administrated into the body by various routes of administration; such as enteral route (oral, sublingual and per rectum), parenteral route (intra muscular, intra venous...), inhalation and topicalto securely achieve its desired therapeutic effect. Each route has advantages, disadvantages and precise purpose. Route of administration is the major aspect of drug delivery

as it is responsible for drug accessibility and treatment (Moeller and Jorgensen, 2008; Prajapat et al., 2017). The route of administration and drug bioavailability are the most important factors in drug delivery. Therefore, the route for drug administration depends on the area of the body needed treatment, the way drug works in the body and the drug formulation.

The release mechanisms for drugs are diffusion, degradation, and swelling. Furthermore, the drug delivery technologies amend drug release profile, distribution, absorption, and elimination, in addition to patient convenience and compliance. Drug release has been an important topic in pharmaceuticals and therefore there is a lot of determinations in such area to develop a targeted delivery in which the drug is only active in the target area of the body, sustained release formulations in which the drug is released over a period of time in a controlled manner from a formulation, and methods to increase survival of dosage which must pass through the stomach environment. Through the development in material design and engineering, novel materials with increasing complexity and more functions have been introduced into the development of drug delivery procedures and systems.

1.2 Routes of Administration

1.2.1 Enteral

Enteral route comprises the drug travelling through human gastrointestinal tract. This route includes the stomach, oesophagus and the large and small intestine. The three enteral routes are Oral, Buccal & Sublingual, and Rectal.

1.2.1.1 Oral

The drug can be administered in different forms. It can be either liquids, tablets, capsules or chewable tablets. The drug typically pass through the mouth and the stomach and greatest amount of drug is absorbed in the small and large intestines. The drug then travels through the intestines and is delivered to the liver where it becomes metabolised, which limits the amount of drug that flows through the bloodstream and to the intended target site. When the drug is ingested orally other drugs and food in the GI tract can affect the rate of absorption of the drug. This route of administering the drug is the most convenient and usually the least expensive and that is why used the most.

1.2.1.2 Sublingual and Buccal

The dosage form can be placed between the teeth and gums (buccally) and under the tongue (sublingually) which can then be disintegrated or dissolved and then be absorbed into the blood stream through the small blood vessels which are situated under the tongue. The intended purpose of these forms is not to be swallowed. This route often gives a rapid absorption rate and can be rapidly enter the systemic circulation.

1.2.1.3 Rectal

Drugs that are given orally can be prepared and administered rectally as a suppository. The drugs are incorporated in a waxy material that liquefies or dissolves when it is placed in the rectum. Due to the rich blood supply and thin walls of the rectum the dug is readily absorbed. People who suffer from nausea when taking medication orally are often referred to taking suppositories instead to overcome this.

1.2.2 Nasal

A drug can be absorbed through the thin mucous membrane by breathing the formulation which leads to the nasal passages. To go through this route, the formulation should be transformed into atomised droplets in the air. The droplets are then absorbed through the nasal passages and then the bloodstream. Drugs moving through this way normally feel the therapeutic effects quite quickly. Some drugs used in this way can irritate the nasal passages.

1.2.3 Inhalation

The drug introduced to respiratory tract and which is containing of a complex selection of organs, tissues and cells. The drugs need to pass through the trachea and reach into the lungs. Depending on the particle size and shape, it determines how far the particles will travel in the lungs. Smaller particles usually travel further which increases the likelihood of the drug being absorbed in this area and enter the blood stream.

It is likely that specialised equipment will be needed to assist to deliver the drugs through this route (Thomas, 2013). Generally, this way is for drugs that are specifically act on the lungs.

1.2.4 Parental

Parenteral route is used by means of an injection through several routes:

1.2.4.1 Subcutaneous (under the skin)

The drug injected under the skin below dermis and epidermis, where the fatty tissues are, by injection. The drug then moves through capillaries and reaches the systemic circulation. However, there are not many blood vessels present in the subcutaneous area, therefore, it is usually used for slow or sustain drug absorption.

1.2.4.2 Intramuscular

Drugs given in large doses directly into the muscle. The absorption of drug is higher than subcutaneous tissue as there are larger and more blood vessels present in this area. The site of administration determines the volume of injection which is limited between 2 and 5 millilitres.

1.2.4.3 Intravenous

The drug is inserted directly into the vein using needle or tube as injection or infusion. This can be done as a single dose or continuous dose. An infusion is an example of a continuous dose and relies on gravity for it to be administered as well as infusion pumps which is pumped through flexible tubes known as a catheter. The purpose of this method is to avoid irritating solution which could cause discomfort intramuscularly and subcutaneously. It has been described to be the best to deliver a precise dose of medicine in a short amount of time and can be easily managed. This is the most continent in emergencies such as a stroke or poisoning. Furthermore, some drugs need to be given intravenously to avoid breaking them by enzymes in the stomach or liver.

1.2.5 Topical

Drugs are placed on the skin which is normally the area where the drug is needed to be active in and is used mainly on to treat superficial skin disorders, such as eczema, psoriasis and skin infections (fungal, bacterial and viral) as well as itchy and dry skin. The drug can be incorporated with different inactive substances into many different forms of topical formulation, such as a gel, solution, cream, ointment, lotion and powders.

1.2.6 Vaginal

Drugs in the form of a tablet, gel, solution, cream, suppository or ring can be given vaginally. The vaginal wall is the route where the drug can slowly absorb through. Oestrogen is usually given through this route to relieve vaginal symptoms such as, redness, dryness and soreness during menopause.

1.2.7 Ocular

Drugs can be incorporated with inactive substances to make a gel, liquid and ointment to treat eye disorders like conjunctivitis, glaucoma and injuries by applying them to the eye. The drug can be absorbed quickly through the eye if good and long contact before it runs off the eye. Prepared drugs in form of ointments and gels have a better retention time when being in contact with the eye as it remains in contact with the eye for a more significant time. Ointments and gels can commonly cause blurred vison.

1.2.8 Transdermal

Transdermal drug administration has been developed to overcome the problems that associated with the conventional formulation such as oral and parenteral. It provides sustain and steady delivery through the skin into the blood circulation. Drugs can be prepared as a patch and be attached on most places on the body. Formulation for these patches usually prepared with adhesive and enhancer and therefore help enhances the penetration of drugs through the skin into the bloodstream. The advantages of a patch would be slow drug release; therefore, a steady and continuous drug supply will be available for over a few hours to days and even longer. This allows for a constant level of drug in the blood. Drugs that are eliminated from the body quickly, patches are the best way to be delivered because if this drug was in any other form would have to be taken at regular intervals and frequently. On the other hand, patches can cause irritation to the skin. The drawback of this method of drug delivery is that the drug is really limited on how quick it can penetrate the skin and also it limited to small doses daily (Nair et al., 2018).

Table 1. 1 Routes of drug administration are described in the table below.

Route	Description	Advantages	Disadvantages
Enteral	Placement of drug directly into the stomach or intestine	Economical, convenient	First pass effect, drugs destruction by digestive liquids
Buccal	Held inside the cheek (buccal area)	Avoid first pass, rapid absorption, drug stability	Inconvenience, dose limit, possibility of being swallowed, irritant drug can't be given.
Inhaled	Breathed in to the longs	Due to high surface area therefore, fast absorption	Drug particle size and patient correct use of inhaler play part in bioavailability of the drugs
Injection (intravenous, intrathecal, intramuscular and infused)	Inject into the vein, muscle, or spine	complete administered dose reaches the systemic circulation without delay	Needs a utility equipment (e.g. cannula) Distressing especially for children Local reactions may appear by intravenous injections
Rectal	Inserted into the rectum	Excellent absorption, Hepatic first pass metabolism is avoided.	Some patients do not like suppositories
Oral	Swallowed by mouth (tablet, capsule, or liquid)	Easy to use Favoured by patients Slow-release	Not suitable for uncooperative patients Administered orally drugs usually absorbed slowly Stomach acid degradation and enzymes affect absorption
Subcutaneous	Under skin injection	Preferred for drugs with a low oral bioavailability Onset is faster than the above routes Can formulate to have very long duration of action	Injections hurt, cause bruises and frighten children and needle phobic

Transdermal	Placed on the skin (e.g. patch)	Avoids first pass and interference with gastric and intestinal fluids, once a day administration	Possibility of local irritation, Drugs with hydrophilic structure having a low penetration through the skin, cannot achieve high drug level in blood
Otic	Drops into the ear	Less invasive, safe, and highly controlled system	Anatomic obstruction of the window membrane, loss of medication, indistinct pharmacokinetic profiles.
Ophthalmic	Eye drops, gel and ointment	Easy to install, non-invasive, long contact time	Blurred vision,
Nasal	Into nose by spray or pump	Drug first pass avoided, gastrointestinal tract fluid is avoided, rapid drug absorption, rapid onset of action,	Relatively inconvenient, smaller absorption area compare to gastrointestinal tract.
Topical	Applied on the skin	Patient satisfaction Non-aggressive Easy to apply	Absorption via skin is very slow especially for drugs with high molecular weight and poorly lipid soluble.
Sublingual	Held under the tongue	Quick termination, first pass avoided, economical	Large quantities not given, few drugs are absorbed, irritation of oral mucosa.

1.3 Drug delivery: Past, present, and future of medicine

While conventional drug administration systems such as oral ingestions, eye drops, transdermal delivery and intravenous injections significantly impact on the control and treatment of diseases, but the on-going development of new administration route with better effectiveness and targeting biological therapeutic has direct and motivate scientists for development of smart therapeutic systems (Yi and Kosel, 2017).

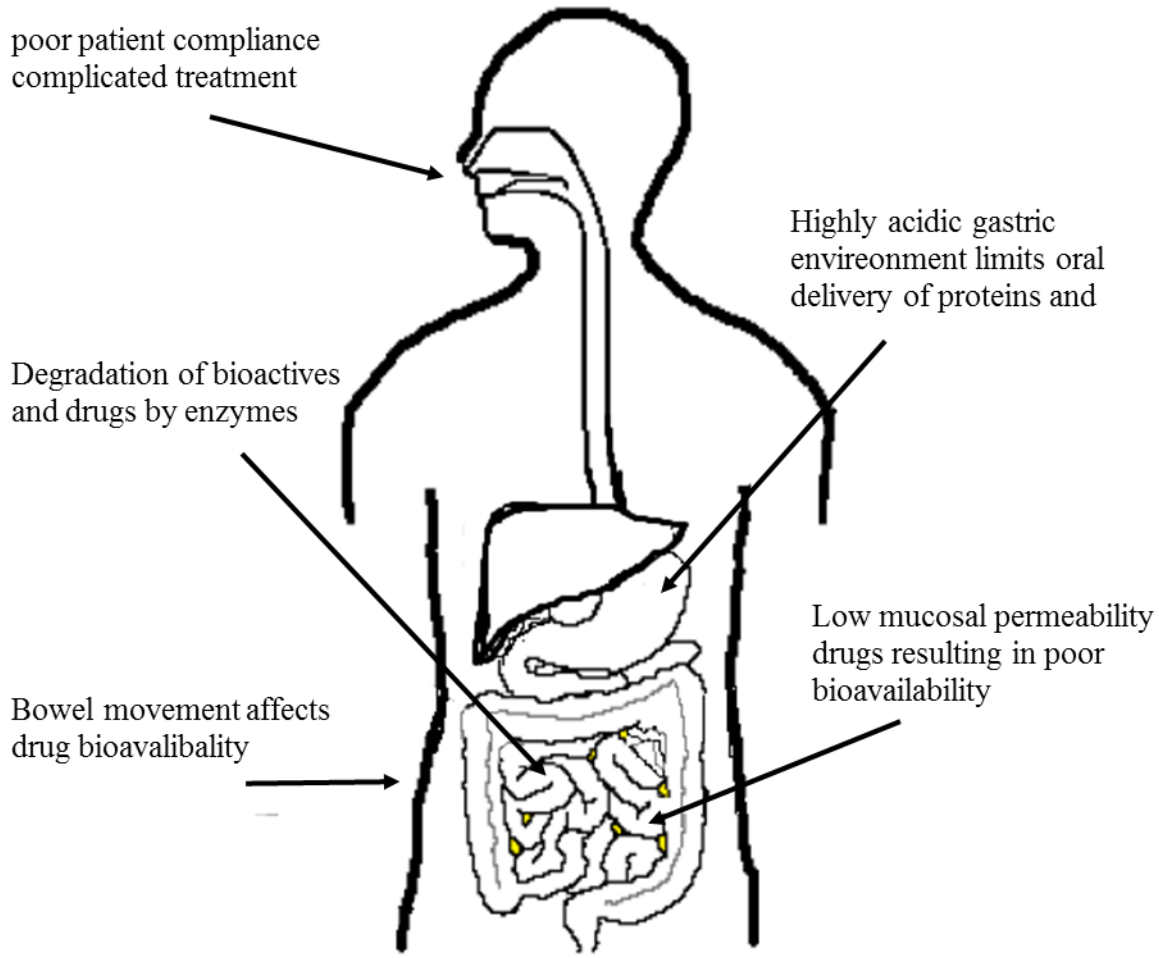


Figure 1. 1 Schematic map of human organs which interfere with drug bioavailability.

Using specific carriers e.g. matrix materials were the one of the earliest progress in the drug delivery methods which aggravates release of active in spatial and time-based manner. This can be accomplished by having it as an implanted reservoir system or pulsatile fashion.

However, these methods are not without any drawback and many concerned such as specific target, bioavailability and intracellular need to be specified before any clinical commencements (Langer and Peppas, 2003). However, polymers proven that have vital part in moderating recent drug delivery systems. These systems give high therapeutic effects and play key role in newly drug delivery development which is not comparable with conventional dosage forms e.g. those which have been used for over the last four decades.

Polymers play a big part in the manufacturing of pharmaceutics. Polymers action comprises of viscosity and flow modifying in liquid dosage forms and as binder in tablets. They are in use to improve the stability of drugs and used as film coating to mask the unpleasant taste of dosage form. Moreover, polymers help to modify drug release kinetics
(Schwarzl et al., 2017). Application of polymer in drug delivery has been growing very fast and extended to very more complicated dosage form systems. As can be seen, that vaccine with polymeric micro particles has been developing to target specific area in the small intestine (Tawde et al., 2012). Adding to this, improvement and development of nano-size chitosan particles which could improve sustain release of promethazine which indicate encapsulation efficiencies of 99.7± 0.06% (Elwerfalli et al., 2015). Polymers in drug delivery has influence the transdermal drug delivery and rapidly growing as well. As in some formulation indomethacin has been combined into conductive polymer (polycarbazole) film. This enhance drug penetration control and modulation through influencing cross-linking ratio and an external electric field; giving rise to a firm controlled transdermal patch system (Thorngkham et al., 2015).

As polymer application and the development continue, for specific target the type of polymer become more vital especially for some delivery systems functionality and structural property is important to be concerned. Polymers are either synthetic or natural. Natural polymers are attained from freely existing sources, which are typically water based and can be extracted e.g. cellulose, agar, chitosan, collagen, silk, DNA, and other proteins. But synthetic polymers are attained form petroleum oil and consequently modified by chemical methods. Examples of synthetic polymers are as polylactic-co-glycolic acid (PLGA), polycaprolactone (PCL), polylactic acid (PLA) andpolyvinylpyrolidone (PVP) polyethylene (PE), and polyester. There have been many developments in biodegradable synthetic polymeric recently which direct into development of e.g. polyglycolide (PGA), PLA, PLGA, and polydioxanone (PDA)). These new inline synthetic polymers shown an ideal member for drug delivery family and validate

acceptable *in vivo* and *in vitro* properties (Gajendiran et al., 2017). Overall, all polymeric biomaterials which are used in drug delivery are endangered to be inspection and effected by surrounding hot environment. In several cases, the reliability of the polymer for intended use has been compromised due to biodegradation. Adding to this, it is extremely important to allow non-toxic removal of the material. As a result, natural polymers (e.g. collagen, gelatine, hyaluronic acid, elastin, aginate, chitosan, heparin, chitin and chondroitin) became more favourable and frequently used for different aspect such as healing the wounds and burn and also developing renewing medicine applications (Sensharma et al., 2017; Murdock and Badylak, 2017; Gajendiran et al., 2017).

However, synthetic materials such as PLA, PLGA and PCL also possess very similar properties which have driven their utility in numerous drug delivery and biomaterial applications (Sensharma et al., 2017).

1.4 Buccal delivery

The development of new drug involves high amount of cost including money and time therefore, it is essential for pharmaceutical industry to reassess delivery option to improve the drug efficacy that have already been accepted. On the other hand, oral delivery is the preferred route for drug administration due to low cost, patient compliance and easy to administrate. But, the degradation of some drugs such as peptide and proteins from gastrointestinal (GI) and hepatic first pass metabolism barriers limits the administration (P. M. Castro et al., 2015). As a result, mucosa was considered as promising site of interest for drug delivery. These include oral cavity, ocular, vaginal, rectal, nasal, and mucosal lining. Amongst these, due to physiological feathers and patient compliance, oral cavity attracts consideration for drug administration (Deepak, Goyal and Rath, 2018; Jug et al., 2018; Lai et al., 2018).

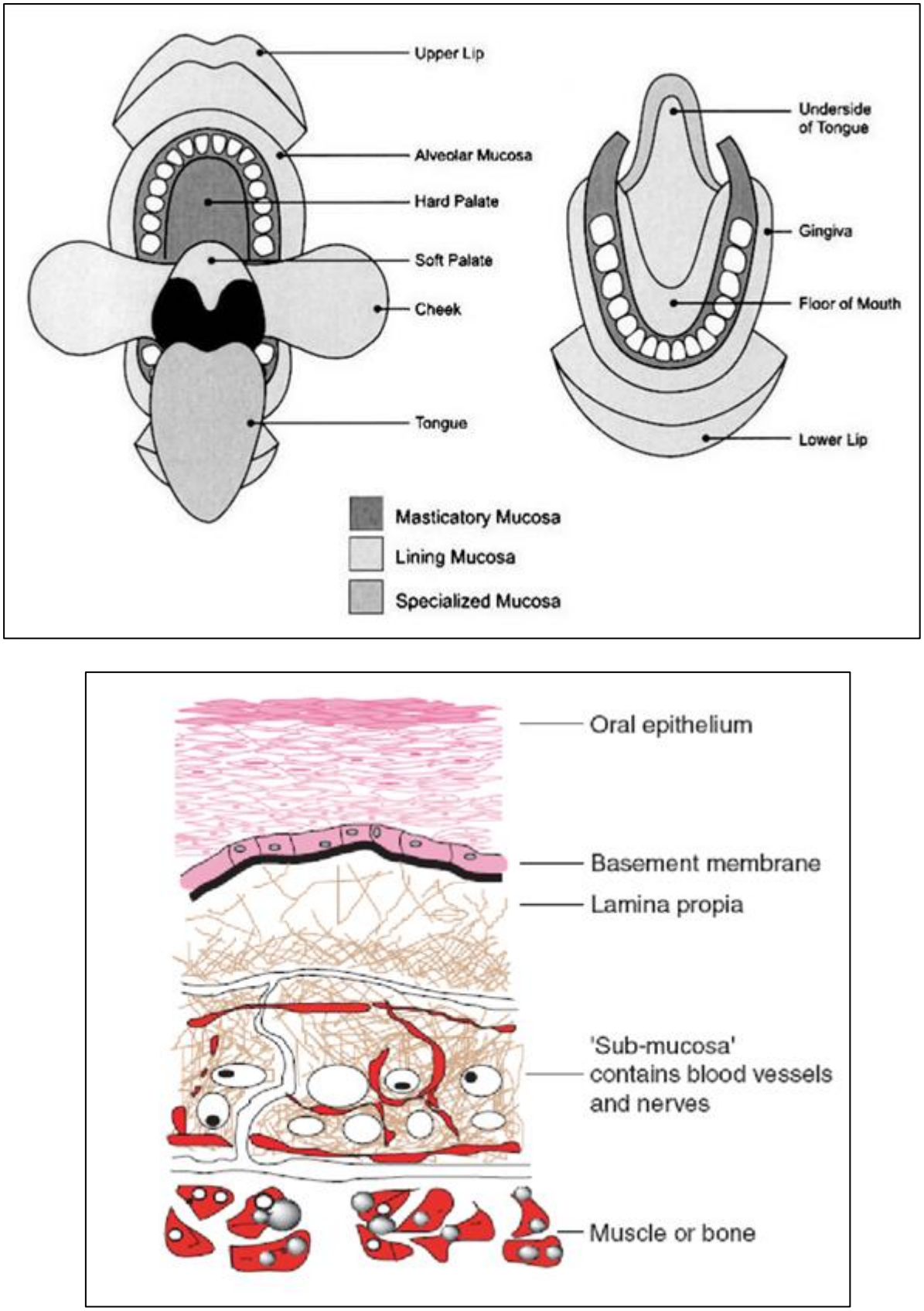


Figure 1. 2 Schematic mouth lining mucosa (top) and schematic diagram of buccal mucosa (bottom) (Patel, Liu and Brown, 2011)

Because of rich vascularisation of oral mucosa and high permeability to very much of drugs, buccal route become an important route of drug administration as an attractive alternative to the oral and parenteral routes for systemic drug delivery (P. Castro et al., 2016; P. M. Castro et al., 2018; Morantes et al., 2017; Mahmoud et al., 2018). It has high total blood flow which ensures systematic bioavailability, helps overcome early drug degradation due to pH and enzyme activity of the gastrointestinal tract (GT), avoids active drug loss due to first-pass hepatic metabolism (Fonseca-Santos and Chorilli, 2018). Therefore, it is essential for the improvement of the therapeutic benefit of many formulations (Russo et al., 2016; Nafee et al., 2003).

Buccal administration route is an alternative to oral route. The buccal drug delivery permits the drug to be delivered across the oral mucous membrane through a passive diffusion process where the drug enters the blood stream directly through oral cavity (P. M. Castro et al., 2018). Buccal drug delivery can improve the absorption of many formulations by avoiding specific problems associated with the drug such as low absorption, decomposition, gastro intestinal irritation, pH problems, due to first pass metabolism in the liver after absorption to the bloodstream and short half-life frequent dosing (Fonseca-Santos and Chorilli, 2018).
It is suitable to be used for treatment of diseases in which prolong release of drug is needed. Also, drugs which cannot be administrated through oral route due to their poor absorption, degradation or high molecule weight, are of interest to be used in buccal route (P. Castro et al., 2016). Preparation of appropriate excipient for the buccal delivery systems could give us a non-irritant and safe dosage in the mouth with sustained-release. However, the main disadvantages of this route are comparatively small surface area of oral mucosa, uncontrolled swallowing and salivary flow and consequently loss of drug. Therefore, the challenge for the success of buccal release is the residence time and maintain dosage contact with the membrane of the dosage forms in the buccal environment which allow the drug act at the buccal site.

A buccal area is the inside of the cheek, plus an area between gums and upper and lower lips (Kraan et al., 2014). Buccal delivery system can be used for either topical or systemic drug delivery. Buccal lining is 500-800 µm in thickness and covers 1/3 (100cm^2) of total oral cavity surface (Sattar, Sayed and Lane, 2014; Fonseca-Santos and Chorilli, 2018). Oral cavity epithelial mucosa differs according to tissue function and is a barrier to lipophilic drugs. Submandibular, sublingual and parotid glands produce saliva liquid. Saliva is distributed over

the mouth and cover the epithelial cells. Saliva comprises of mucus, enzymes, proteins and minerals (Hannig et al., 2017; Fonseca-Santos and Chorilli, 2018; Malallah et al., 2018).

Buccal mucosa has been widely explored recently, though, its potential as drug delivery route has been known for centuries. Nowadays researchers coming up with novel drug delivery systems to provide better bioavailability and convivences, such as films, patches, adhesive tablets and nanotechnology-based systems.

Transcellular and paracellular are the major drug transport across buccal mucosa (Abd-Elbary et al., 2016). It has a rich blood supply which facilitates the direct entry of drug molecules into the circulation system and therefore improves the bioavailability of drugs (Ansari, Sadarani and Majumdar, 2018).

It is important that the excipient (polymer) has the ability requirement for buccal administration which is optimal polarity and fluidity. This is to make sure the polymer is sufficiently wetted by the mucus and to consents common adsorption and the action of penetration between polymer and mucus to take place within a realistic time schedule (Ikeuchi-Takahashi, Sasatsu and Onishi, 2013).

Because buccal has a superb accessibility of the absorption area, toughness, and the prospects of high patient acceptance, and permits drug absorption to be terminated in the case of any adverse reaction will direct to a regular attention to this area for drug delivery (Nazari et al., 2017). The best buccal dosage form must have the following prospects. It should a) stay in its position in the mouth for intended hours b) drug release should be in controlled manner c) unidirectional drug release toward the mucosa.

Therefore, to achieve these requirements for a buccal dosage using right mucoadhesive polymers as excipient is important. The mucoadhesive should be able to control drug release as well. (Govindasamy, Kesavan and Narasimha, 2013)

1.5 Processing Technologies

Due to favourable physical properties such as lower density, ease of modification, measured degradability and minor environmental effects, polymers gained attention in pharmaceutical field (Vedadghavami et al., 2017). The new development in drug delivery systems are as result of advances that gained from polymer science. The need for new and more effective

biomaterials has helped for development of various polymers, especially during the last decades. The development cost and property of the biomedical are the important aspect for manufacturing and the development of delivery systems. Process-ability, impact strength, biodegradability and biocompatibility are other aspect to be considered for biomedical applications. Furthermore, the biomedical need to be processed and explored by several technologies such as spray drying, microfluidics, super critical fluids, soft lithography and freeze drying. This allows designing, analysing and controlling the process parameters and manufacturing protocols and aiming at understanding the materials by monitoring them in a timely manner and therefore reducing the process time and enhancing consistency and the quality of final product. This is important as choosing and designing the right polymer for the right technology is a challenging task. Desire polymer should have mechanical, interfacial, chemical and biological function of purpose of use (Pillai and Panchagnula, 2001).

1.6 Spray drying

Spray drying is a continues process of drying solutions, suspensions and liquids. It is a simple, rapid, easily scalable and reproducible technology which is well accepted in industry (Sarrate et al., 2015), (Paudel et al., 2013). It is a constructive one step process that compromises the transformation of a material from a uniform solution or suspension state into a fine dry powder particle. This achieved from atomisation through a nozzle into a hot drying gas medium (Seremeta et al., 2014). The formulation loses moisture quickly and dry when encounter with stream of hot gas. By changing the parameters for example formulation viscosity, rate of feeding or atomization pressure, it is conceivable to modify the produced particle size (Hede, Bach and Jensen, 2008). In addition, the final product (powder) produced have practically consistent characteristic (Davis and Walker, 2018) and (Davis and Walker, 2018). There has been such a development and improving in all parameters of this technology. These include air dispenser, air inlet control, temperature and energy optimisation spatially for sensitive drugs, and overall the sanitary aspects of the complete system. Further system control could be developed and adjusted for inline and online control and measuring the final product quality. Spray drying provides advantage of incorporating several excipients into one formulation and many of parameters can be adjusted to produce and design the powder for intended use for instance powder for lungs delivery. This system was used for numerous drug delivery development systems and used for preparation of several pharmaceutical products such as

tablets, microencapsulated ingredients and respirable powders. This method of drying is favourable not only because of producing consistent particle size and morphology but also preparing many heat sensitive materials.

This method used for the encapsulation of didanosine (water-soluble anti-retroviral used in the treatment of HIV) as for the biocompatible synthetic PCL which produce particles with range diameter size of about 36-118 µm and encapsulation efficiencies ranging from about 60-100% (Seremeta et al., 2014; Sosnik and Seremeta, 2015).

1.7 Microfluidics process

Microfluidic processes and capabilities are multidisciplinary field where small amount of fluids are mixed, separated and moved forward and therefore, accurately controlled and handled in channel of between 10-100 nanometres. These technologies employ several cooperative micro-engineered platform technologies which incorporate with micro-channels, micro-membranes, modified surfaces and immiscible liquids at accurate interfaces. A typical microfluidic set up includes syringe pump, and tubing which connected to a microfluidic device. The device usually residences on top of a microscope slide.

In the field of drug delivery, the microfluidic technique can fabricate monodisperse nanoparticles with uniform morphology and size, which is a great advantage compare to other method of nanoparticle preparation (Zhao et al., 2017; Costa et al., 2017; Balbino et al., 2017). The key benefits of microfluidic systems rotate around implementing operation systems that can be used to require for a laboratory in a micro size device that can be reproducible and controlled. Particles obtained from microfluidic devices display little polydispersity. Moving away from conventional types of microfluidics (i.e. for the preparation of near uniform particles or droplets, when two immiscible liquids meet at micro-interfaces) (Björnmalm, Yan and Caruso, 2014), several other therapeutic forms have emerged from microfluidic principles. Recent developments have shown microfluidic engineering of drug particle eluting microbubbles and drug particle generating droplets (Kucuk et al., 2014). However, the scale of operation usually becomes a bottle neck for such systems. A variety of microfluidic devices have also been explored for other biomedical applications, which enable on-chip point of care diagnosis as well as real time monitoring using minute quantities of body fluid [.For example, biomarker detection in disease diagnosis for cancer, in comparatively rapid fashion, has been demonstrated (Y. Wu et al., 2014).

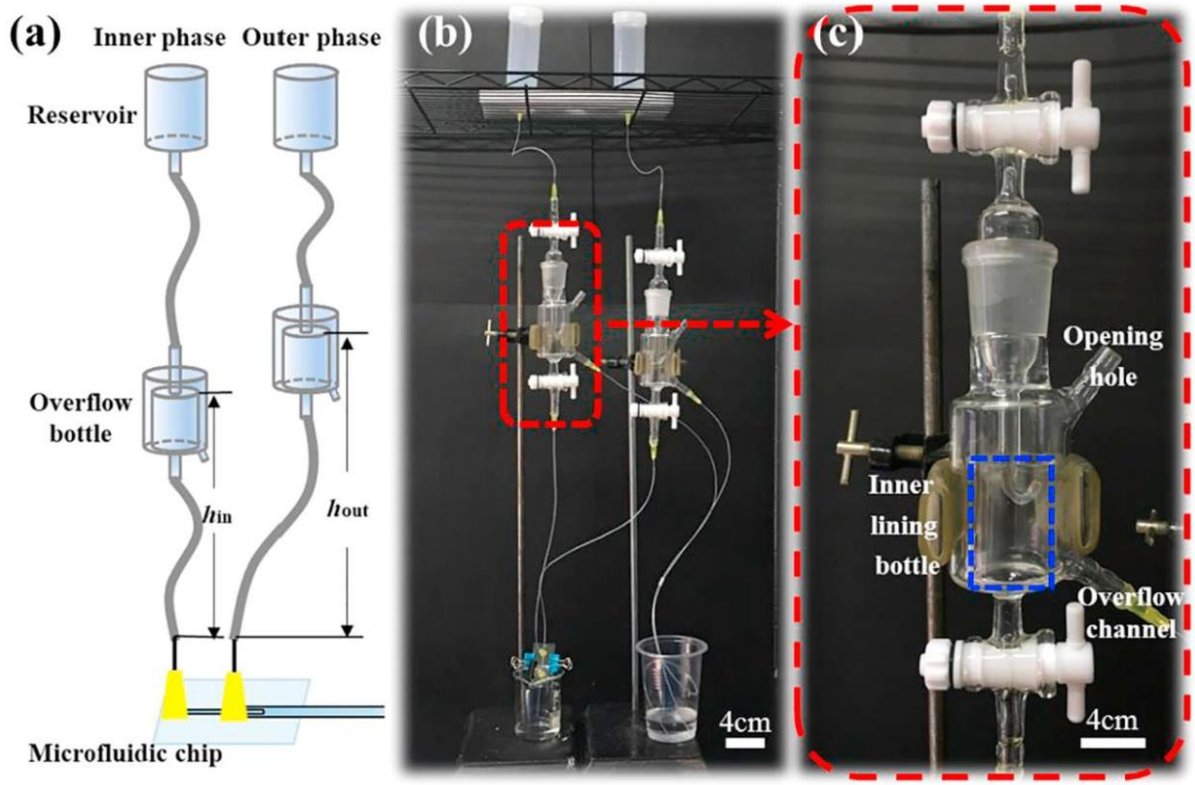


Figure 1. 3 Simple two unite gravity driven microfluidic system: a) scheme of microfluidic system setup; b) image of overflow system; c) closeup image of the overflow bottle. (Gao et al., 2019)

1.8 Supercritical fluids (SCF)

Engendering of particles in drug delivery which gain control of particle size and polymorphic purity can be challenging. Particle size is important by means of it is affects the path of drug delivery and as a result the targeted area. Ideal particle size for intervenors delivery should be around 0.1-0.3 µm. for inhalation delivery 1-5µm. and for oral delivery 0.1-100 µm. regardless of these facts, control and modifying the particle size cannot always be achieved by the conventional micronizing such as milling and grinding or jet milling techniques which does not produce uniform particle size distribution (Tabernero, Martín del Valle and Galán, 2012). Supercritical fluid technique has been employed for the formation of verity particle sizes. It has been used to produce ultrafine particle and has great advantages over conventional size reduction methods. The process based on the solvent-impact phase separation in which the addition of gas causes the precipitation of particles (Yeo, Choi and Lee, 2000; Reverchon, 1999).

Another alternative strategy in polymer process engineering technology is the supercritical fluid technique that is classified for crystal and particle engineering for developing cosmetic, drug delivery and pharmaceutical materials (design of particles, particle size control or polymorphic purity) for which it has shown great promise. Supercritical fluids are liquids and gases deployed at pressures and temperatures above their critical points (Figure 1. 4). At critical point the change in volume between the two phases goes to zero and above the critical point there are no longer noticeably defined phases. Therefore, this means that a supercritical fluid cannot be compressed with decreasing volume and increasing pressure into liquid at constant temperature. Also, a supercritical fluid will not evaporate to vapor by reducing the pressure and increasing volume.

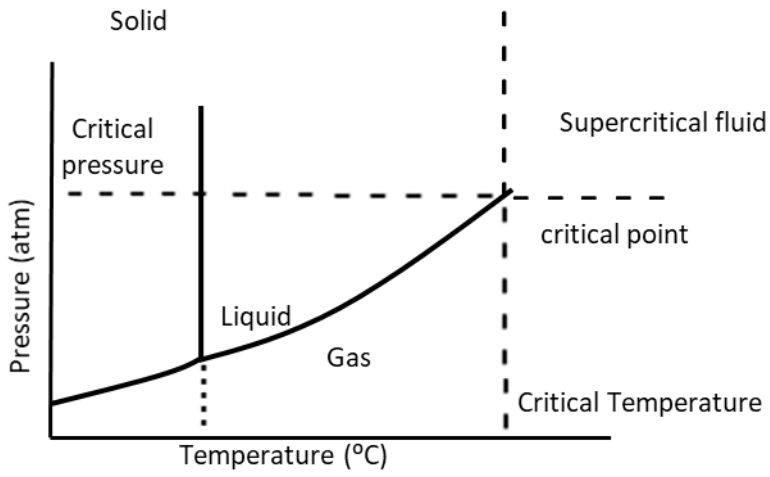


Figure 1. 4 Phase diagram of physicochemical properties of a compound.

This process can be driven using a selection of materials and co-process which give rise to particle size control engineering. The sub-processes developed here can involve rapid expansion, re-crystallisation, solvent extraction and gas saturated suspensions, with resulting particle size ranging from a few hundred nanometres to several hundred microns(Yeo, Choi and Lee, 2000; Montes et al., 2016; Djerafi et al., 2017).These methods have been favourable due to their green (environmentally favourable) nature.

1.9 Soft Lithography

Over the past years, microfabrication technology has gained great interest and development for variety of health care related products. A lot of research is done on diagnostic tools and microdevices for therapeutic applications such as implants and drug delivery micropumps. Microparticles made of silicon dioxide and photocurable polymers have been generated using the photolithography microfabrication technique. In this instance microparticles demonstrating low polydispersity and similar surface chemistries were prepared for deployment as drug delivery systems. Nevertheless, there is a limited choice of materials, and in addition some lithographic fabrication method requirements include the utilisation of corrosive etching solutions which may damage delicate or sensitive compounds (Tao and Desai, 2003; Hilt and Peppas, 2005). Micro- and nano-fabrication using an elastomeric stamp, which is prepared by cast molding based on self-assembly and replica (to generate pattern and structures with mean sizes ranging from ~30 nm to 100 µm) is a general method of several non-photolithographic techniques which are termed ‘soft’ lithography. In comparison to non-photolithographic processes, planar and non-planar surfaces as well as 2D and 3D structures can be generated. This can be demonstrated as; microcontact printing (µCP), replica molding (REM), solvent-assisted micromolding (SAMIM), micro-transfermolding (µTM) and micromolding in capillaries (MIMIC). Moga et al. (2013) demonstrated the direct fabrication of biocompatible dissolvable polymeric microneedles on to water soluble substrates (flexible) through a soft lithography method (PRINT - particle replication in non-wetting templates) presenting the ability to penetrate both murine and human skin samples. Polymeric microneedles were prepared from biocompatible and water-soluble polymer, PVP (10 kDa) and rhodamine B dye at a loading concentration of 0.1%. Here, rhodamine B dye was clearly delivered to both selected tissue types (*ex vivo* murine and human skin specimens). Hence, the conclusion stated that such polymeric micro-needle patches can be used to prevent or treat a variety of systemic

and local diseases, including skin cancers, vaccines and other treatments that need routine hypodermic type of injections.

1.10 Spray Freeze-Drying

Spray freeze drying characterised by atomisation of the suspension or solution into the cold gas phase of the evaporation cryogen. During the proses the porous flow chamber is cooled down to the desired temperature usually lower than -50°C using liquid nitrogen. After that liquid feed is atomised into the cryogenic gas within the flow chamber. The result frozen droplets are collected on the exit filter at the bottom. Solidification of droplet starts while passing through the refrigerant vapor above the liquid surface. After that the microscopic droplets which were freeze in the cold air are fluidised inside the chamber until all the moisture has been removed by sublimation under pressure and low temperature (Teixeira et al., 2017; Wanning, Süverkrüp and Lamprecht, 2015). Spray freeze-drying techniques have been developed to avoid high temperature usually associated with other pharmaceutical engineering methods such as spray drying, as this can be damaging to polymeric excipients and the active ingredient. Microencapsulation of proteins in biodegradable polymers is a viable route for their delivery and sustained release which has been demonstrated on the micron scale (Adeli, 2017; Borges Sebastião, Robinson and Alexeenko, 2017). Recently, the spray freeze-drying method has been explored as an alternative to the ionic gelation technique for the generation of chitosan and alginate nanoparticles intended for colonic delivery. In this instance, nanoparticles containing prednisolone and inulin were developed using two separate processes; spray freeze drying using chitosan and spray freeze drying using alginate. Characterisation and assessment of nanoparticles was based on release kinetics of prednisolone and inulin, sensitivity to lysozyme degradation and bacterial and faecal slurry stability. The findings indicate chitosan nanoparticles demonstrated similar particle sizes and structures but with lower encapsulation efficiency for inulin. Moreover, less degradability in the presence of lysozyme and *E. coli* and no degradability by faecal slurry were also shown. In contrast, alginate loaded nanoparticles displayed appreciative encapsulation parameters for both actives, and biological degradability by *E. coli* and faecal slurry was also confirmed (Gamboa et al., 2015; Wanning, Süverkrüp and Lamprecht, 2015).

1.11 Electrohydrodynamic Atomization (EHDA) Technologies

EHDA is a process by which electrical forces are used to atomise liquids. Appling voltage which is the driving force, solution formulation is force out of the nozzle, therefore, producing the formulation as micro/nano fibre or particles depending on the formulations. These structures are currently attracting a lot of interest in the biomedical field, applicable to an extensive range of applications (i.e. drug delivery and biomaterials).

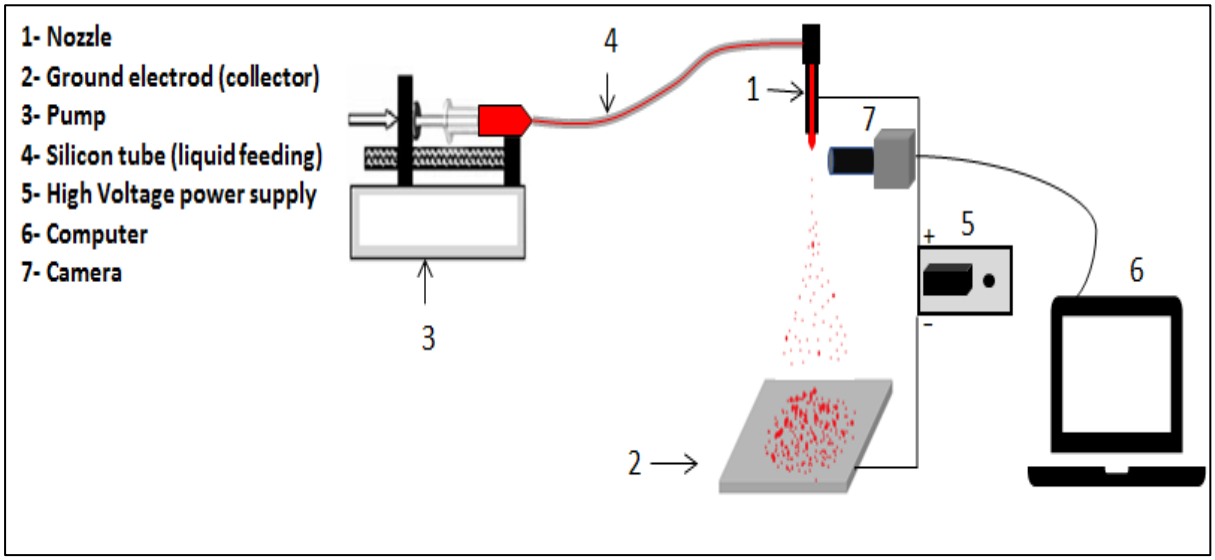


Figure 1. 5 Schematic electrospraying setup

In this engineering technique, small droplets are obtained from an electrically forced (electro) break-up of a moving (dynamic) liquid (hydro) at a controlled flow rate through a conducting capillary needle (or nozzle) maintained at several kilovolts (usually ~30 kV max) relative to a ground electrode. The EHDA process is a well-known yet maturing physical process where a liquid jet (such as drug formulation) breaks up into very small droplets due to electrical and mechanical forces. This is dependent on the strength of the electrical stress and the resulting kinetic energy of liquid jet leaving the nozzle, which contribute towards a variety of spraying (or jetting) modes. Once these droplets are ejected, the base solvent in the liquid formulation evaporates leaving behind, typically, polymeric excipients and drug to form solidified matrix systems on nanometre scales. The formulation or processing liquid in this regard is optimised to engineer surface and dimensional properties of dried structures.

Basic EHDA principles (interacting antagonistic forces during EHDA jet enablement) are usually coupled with formulation material properties (visco-elastic properties) which are often utilised to determine process viability, fibre or particle generation and size of structures. For these reasons, the deployment of polymeric materials (both natural and synthetic) in these processes has been very favourable; to engineer a range of micron scaled drug delivery systems. Several formats of the EHDA processes exist, including electrospinning (for fibre and thread generation), electrospraying (for particle or droplet formation), multiple needle coaxial forming (layered particles and fibres), bubbling (gaseous vesicles) and more recently printing and patterning (ordered architectures). When voltage is applied to the conducting needle, the flowing liquid (formulation for drug delivery) forms a jet at the needle orifice, which breaks up into fine droplets that are electrically charged, therefore enabling them to be guided and focused easily, minimising droplet agglomeration. This technique also allows the generation of near uniform size distributions (once optimised) along with ambient temperature processing, which are distinct advantages when compared to existing pharmaceutical technologies not viable for sensitive materials (e.g. proteins and peptides).

It was William Gilbert in 1600 who observed and record this spectacle. Following observation on electrospraying was by Jean-Antoine Nollet in 1750. But it was in 1900 when the first patented EHDA setup was recorded. Rayleigh made the earliest observations on droplet instability due to electric charges and suggested the conditions for a drop or jet to become unstable, since the stability of the drop or jet is dependent on the balance of forces, i.e. the outward electrical stresses disrupting the drop and the counteracting surface tension forces trying to hold the droplet together. Based on Rayleigh's theories, Zeleny pioneered early experimental studies of EHDA and observed different modes of spraying at the exit of a capillary, which was exposed to an external electric field; able to produce a qualitative description of the process based on specific parameters (flow rate and applied voltage) (Xie et al., 2015).

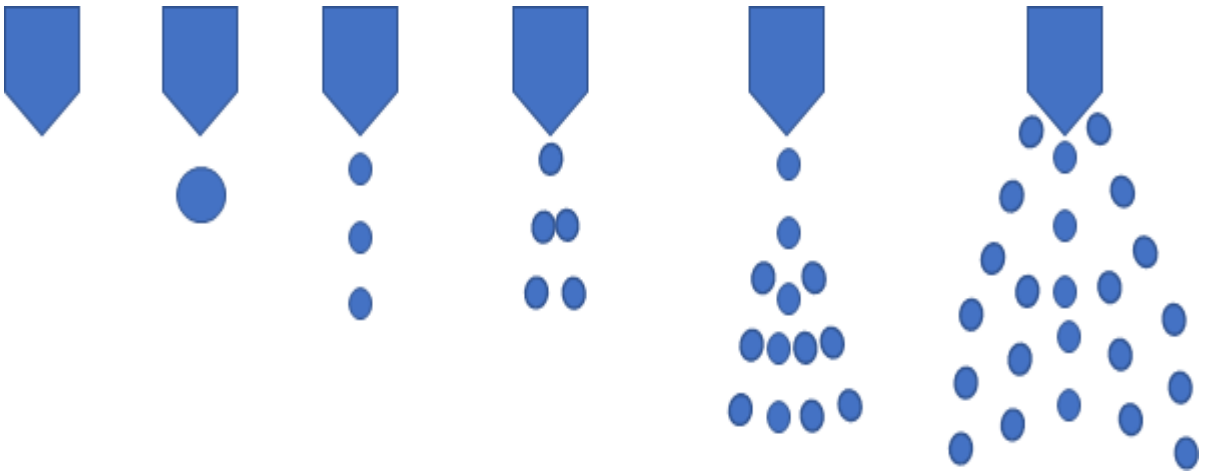


Figure 1. 6 Schematic of droplet in presence of electric field

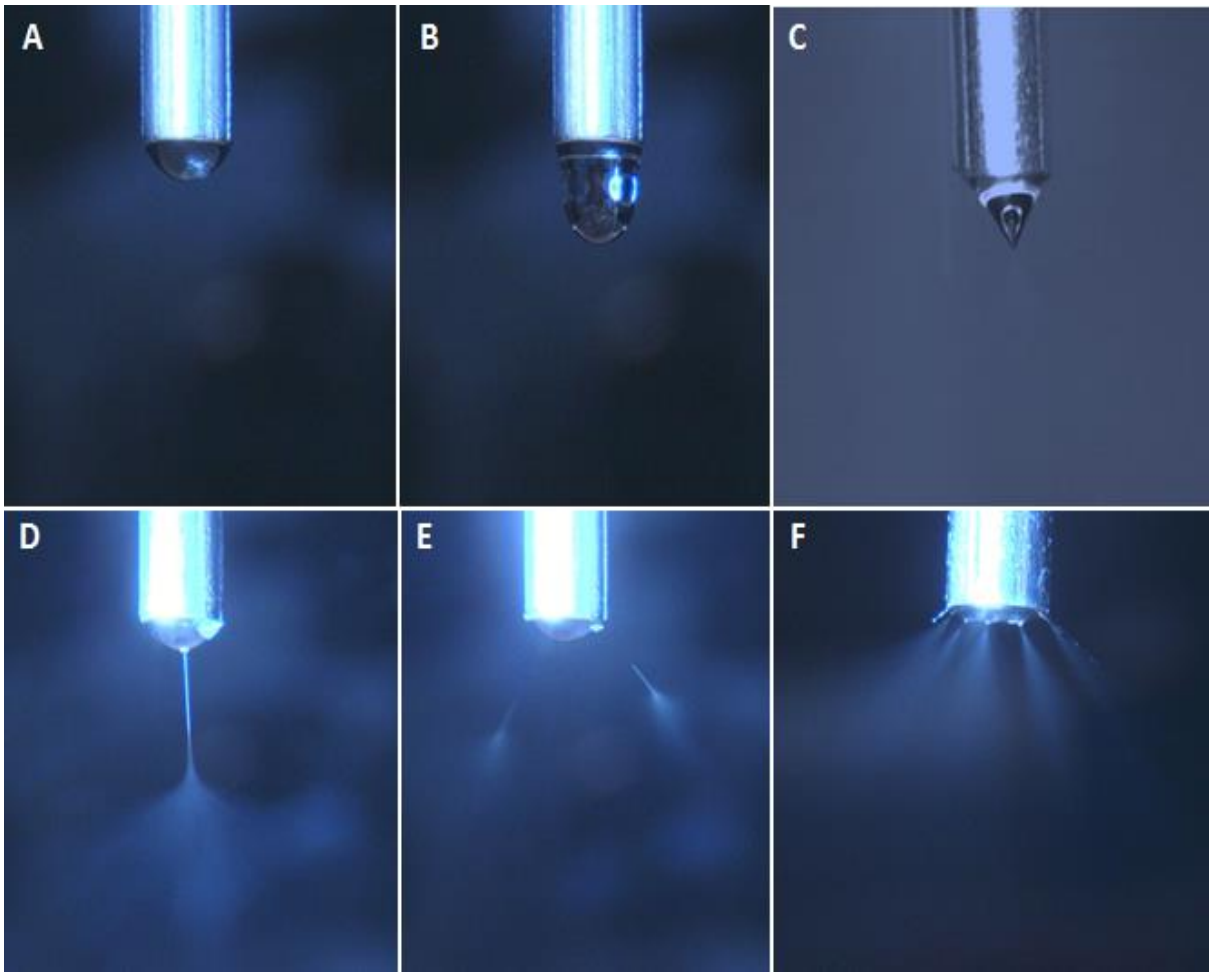


Figure 1. 7 Spraying modes, A and B) droplet, C) shows Tyler cone, D) single jet, E) double jets, F) multi jets

Since the late 20th - early 21st centuries this phenomenon has attracted a considerable amount of interest from both fundamental and application viewpoints, and experimental features in the apparatus used by Zeleny are still being used and developed further by scientists. The production of droplets during electrostatic atomisation can be detected because of applying an electric potential difference between a ground electrode and the conducting capillary/nozzle (used for formulation media flow). Based on the balance of the competing interaction between the electrical force and surface tension, the jet formed from the capillary can break up into droplets in several different ways. Many researchers have discussed the various modes of electrostatic atomization and have classified them according to the properties of meniscus and jet. Most researchers have used the geometrical forms of the jet and meniscus as defining conditions of process viability (Grace and Marijnissen, 1994). These modes are discussed based on two main characteristics; 1-the geometrical configuration of liquid orientated at the capillary needle exit, and 2-the type of jet formation and subsequent disintegration into droplets according to the studies by Jaworek and Krupa (1999) (Jaworek and Krupa, 1999). Based on these criteria, the spraying modes are divided into two groups. The modes in which liquid fragments are ejected from the capillary exit (e.g. dripping and spindle modes) and the modes in which the liquid produces a continuous jet, which subsequently breaks up into droplets a few millimetres from the exit of the capillary. The second group (meniscus and the jet) can be stable, vibrating or rotating in a spiral fashion circulating the capillary; an example is the famous desirable cone jet mode (stable).

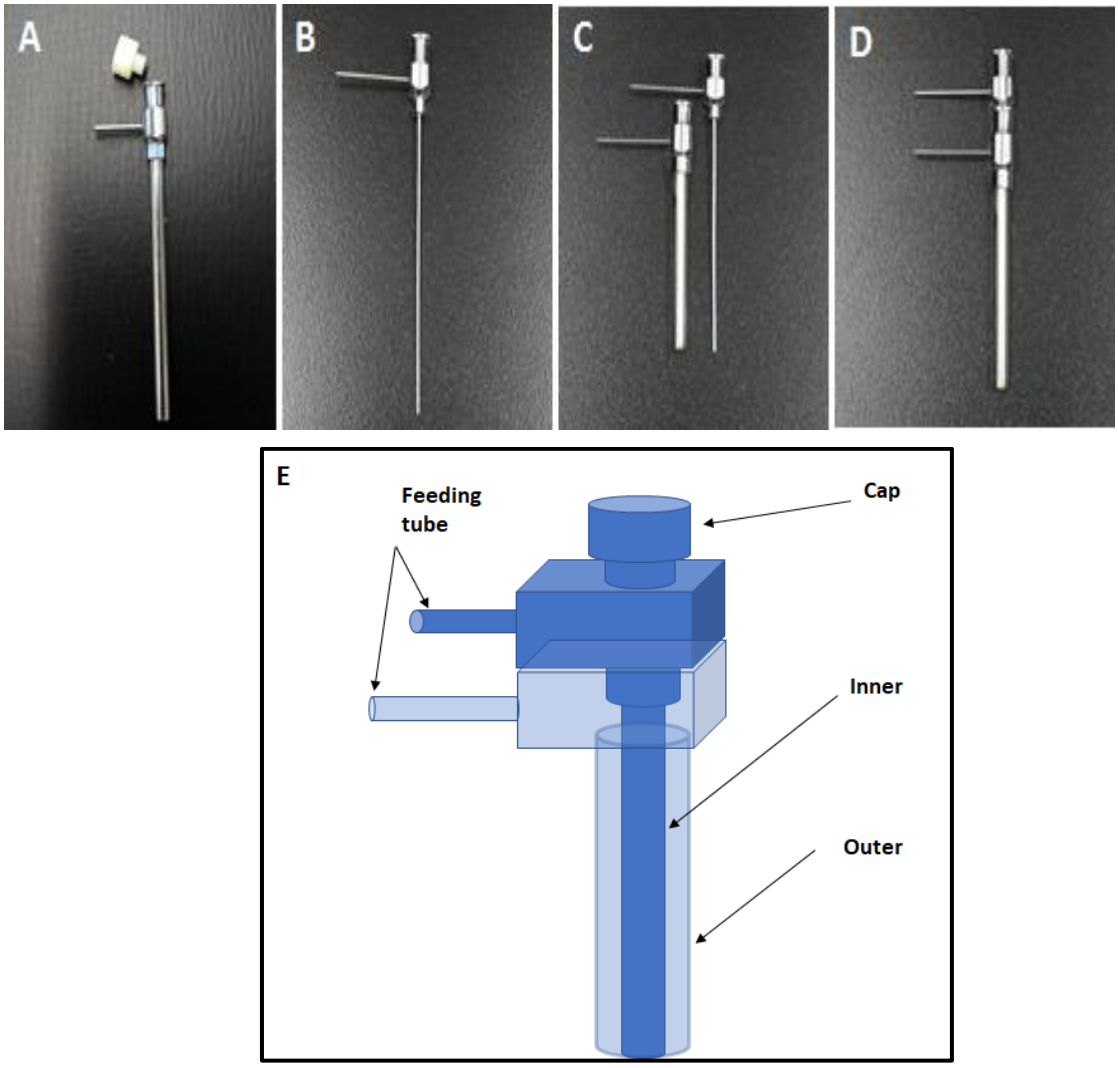


Figure 1. 8 Image of electrospinning needles. A and B) single needle with different diameter, C and D) Coaxial needles system and E) Schematic of coaxial needles system.

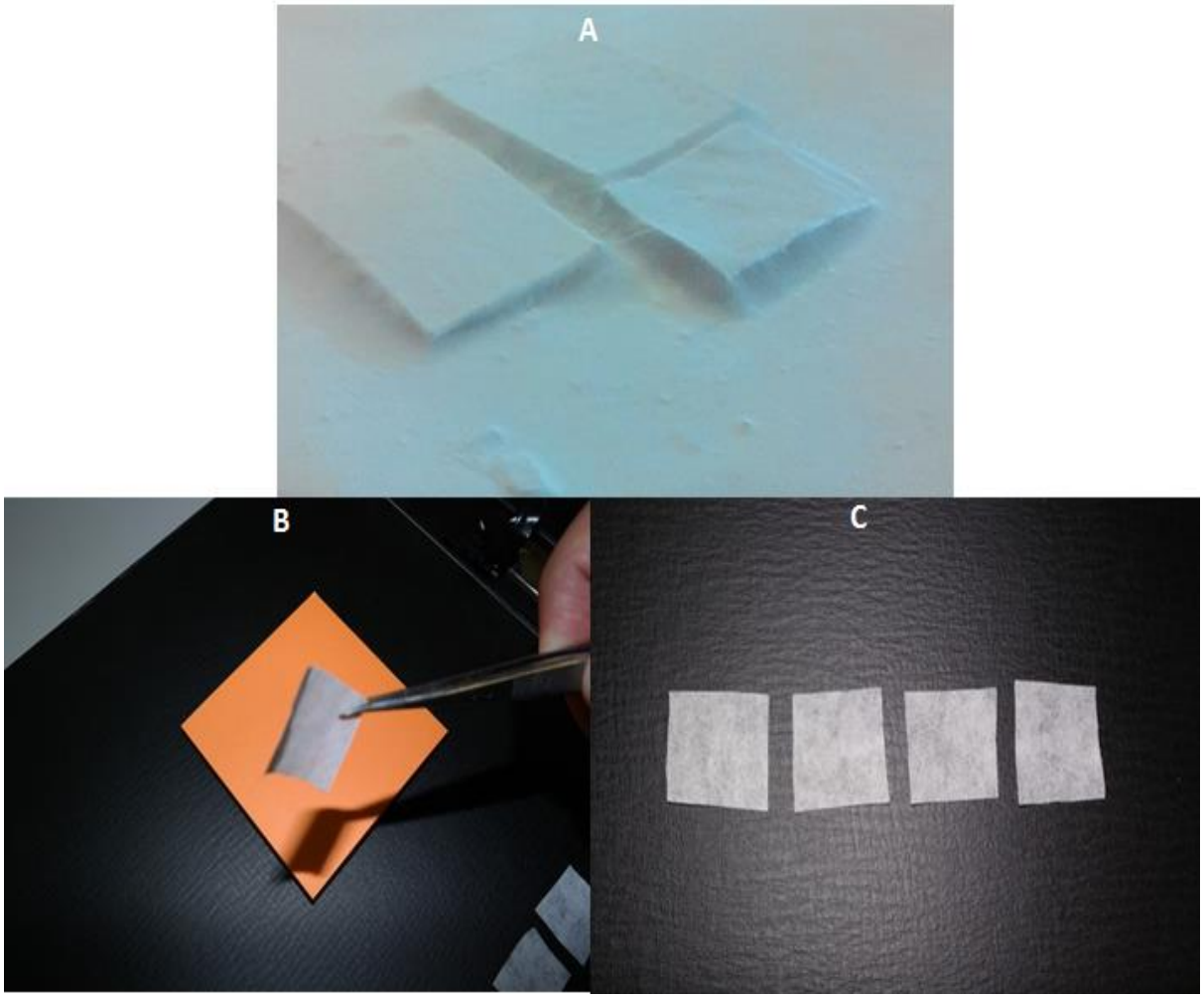


Figure 1. 9 Image of electrospinning products. A) Fibres dispensed on collector surface, B and C) fibres cuts.

1.12 Processing parameters

The key variable in establishing the cone-jet mode is the applied voltage, and the cone-jet mode can prevail within a range of applied voltage values, permitting the establishment of an operating window for stable jetting. The electric field is a key parameter in controlling the process and is dependent on the distance between the needle and the ground electrode. A greater applied voltage leads to a stronger atomisation effect on the liquid (or formulation), which is mainly due to electrical forces produced by this field. At a desirable specific voltage range, the meniscus of liquid becomes conical and stationary and below this the liquid ejection at the nozzle/needle often operates in pulsating mode. Droplet size reduces with an increase in applied voltage and increases with the flow rate, thus it is paramount to select the highest possible value of applied voltage and the lowest possible value of flow rate to achieve the minimum droplet size which correlates to particle diameter once dried. However, the influence of the applied voltage in the EHDA description of a liquid is relatively small when compared with the flow rate, i.e. for highly conducting and viscous liquids the size of electrosprayed droplets from the cone-jet mode are relatively insensitive to the applied electrical field.

A stable cone-jet mode does not exist below the minimum flow rate of every single liquid, as each liquid has a minimum flow rate for EHDA processing. The size distribution generated in EHDA (cone-jet mode) depends on several factors and these include the diameter of the jet, and its resulting break into miniaturised droplets. The process of jet breaks up is mainly due to varicose instabilities at the minimum flow rate. At higher flow rates the charge on the jet surface increases. Above a certain surface charge value, the lateral instabilities of the jet also come into play and are often termed kink instabilities. The size distribution of main ejected droplets also becomes wider based on the magnitude of these instabilities. The minimum flow rate (Q^o) of a liquid sprayed in the cone-jet mode can be estimated by using the equation provided by Ganon-Calvo (1997):

$$Q^o = \varepsilon^\circ / K\rho \qquad \text{Equation 1. 1}$$

Where ε°, K and ρ are permittivity of free space, electrical conductivity and density of the liquid, respectively. The flow rate influences the droplet size and fabrication rate extensively

and it can be adjusted by varying the output of the syringe pump or by changing the cross-sectional area of the tube transporting the liquid. Only within a certain flow rate range does the cone-jet mode form and these limits are generally imposed experimentally. The EHDA process is not stable if the flow rate is considerably low as the liquid volume is not enough to form the cone shape and here the liquid relaxes to form a droplet shape intermittently. Conversely, if the flow rate is increased above a critical value, the impact on droplet size distribution becomes strikingly apparent which yields more polydisperse structures. The process becomes more unstable with increasing flow rate and as a result the polydispersity increases.

Additionally, there are a few more parameters that need to be considered such as the configuration of the needle, the ground electrode (including its shape and movement) and the wettability of the needle. The shape/geometry/movement of the ground electrode (point, ring or plate) can determine the deposition behaviour of resulting droplets, andplays a crucial role (especially with regards to newer technologies involving patterning) in the EHDA process. Also the distance between the needle tip and the ground electrode affects the EHDA process; when the ‘’needle exit to ground’’ spacing is large, the applied voltage boundaries for the various stability parameters are higher.
Figure 1 provides images of typical laboratory set-up of an EHDA system showing (a) key components, (b) spraying during the cone-jet mode, (c) spinning of fibres from an electrically conductive nozzle and (d) a close-up of coaxial EHDA needles. To demonstrate these processes PVP polymer-dye-ethanol formulations were used.

1.13 Advantages and disadvantages of electrospinning process

Numerous methods have been used to manufacture drugs in pharmaceutical manufacturing for different propose such as quality and to lowering the cost of manufacturing. Electrospinning is regarded as a versatile and simple method to generate ultrafine fibres that are between tens of nanometres and several micrometres in diameter using a rich variety of materials that includes polymers, inorganic materials, and composite materials. It is worth noting that electrospinning is a continuous process that results in the formation of longer fibres compared to fibres prepared by other chemical or physical methods. Homogeneous morphologies and structures can produce desired properties by controlling the process parameters and/or designing special electrospinning apparatus.

Electrospinning presently has numerous restrictions. First the variety of polymers used in electrospinning is limited. Second, electrospinning has been implemented at industrial level; however, in terms of producing fibres for the application of filters electrospinning is inferior to traditional methods due to its higher cost to produce fibres with large diameter. In addition, the disadvantages of high cost of production, process controlling make electrospinning more attractive.

Synthetic and naturally occurring polymers have been utilised in both well established and emerging pharmaceutical (and therapeutic) materials and dosage forms. The utilisation of these materials has also been expanded from serving as mere fillers and single function matrix materials to controlled release excipients and multi-functional enabling structures. The latter has been expedited due to further functionalisation or careful engineering. EHDA technologies are robust technological platforms permitted the one-step engineering of a selection of polymers (and therefore integration of their unique properties) on several scales which are able to meet requirements from the past and those currently emerging as future therapies including fibres, nanoparticles and bubbles.

1.14 Process analytical technologies

An optimised electrospinning/electrospraying (EHDA) process can be considered as that which achieves an optimised drug product for the least cost (include time and money). This requires an understanding of the factors affecting each of the controllable parameters in the EHDA process. There is therefore increasing interest in the deployment of reliable, and rapid feed-back technology to aid in the EDHA process control. This could minimise the development time through enhanced understanding of the material attributes of the product and the process parameters required for an optimised process. From this understanding one can develop a control space that will ensure that the product meets its target quality product profile. Monitoring of the control space requires in line QBD tools; process analytical technologies (PAT) which provide feedback control of various critical to quality attributes of the product, such as final fiver diameter size or particle size content.

Process analytical technology (PAT) is an organization for designing, analysing, and controlling manufacturing productions. This is done all the way through a well-timed measurement (e.g. during processing) of essential quality and performance attributes materials

and processes with the aim of ensuring final product quality (Yu et al., 2004). The desired aim of the PAT framework is to design and develop processes that can consistently ensure a predefined quality at the end of the manufacturing process.

In the EHDA area there have been many developments in process analytical technologies to control different aspect of the process (e.g. Tyler cone, jetting, spray and fiber/particle size). These are frequently classified as individual online measurements, which include technologies such as high definition camera, laser particle size measurement, live monitoring of the process.

1.15 Limitations of currently available process analytical technologies

Most of the process analytical technologies are intended for the determination of end of process. Implementation of new technology is needed to the system which could help for optimising the process parameters and as result impact product homogeneity. Moreover, equipment set up, the environment and the electrospinning system itself play part in the final production and end-product results. However, system in en-closed system can have better result.

1.16 Material/Physical properties

The main material/physical properties of liquid (formulation) which influence the EHDA process are viscosity, surface tension, relative permittivity, electrical conductivity and density. Viscosity is a fundamental liquid property playing a significant role in the jet break-up process by influencing the hydrodynamic aspect of EHDA modes, which also impacts on the size of generated droplets, thus the resulting particles after drying. In the EHDA process, an increase in viscosity over three orders of magnitude has a dramatic effect on the size of particles obtained that also leads to an increase in droplet size. Rosell-Llompart &de la Mora (1994) suggested the effect of solution or medium viscosity on resulting droplet size is dependent on a dimensionless parameter π_η in the cone-jet mode, given by:

$$\pi_\eta = \left(\sqrt[3]{\gamma^2}\rho\beta\varepsilon^\circ/K\right)\Big/_\eta \qquad \text{Equation 1. 2}$$

Where η, γ and ρ are the viscosity, surface tension and density of the liquid respectively, and β, ε° and K are the relative permittivity, vacuum permittivity and electrical conductivity of the liquid respectively. They proposed that if π_η is >> 1, the viscosity of the perfused liquid has no impact on the resulting droplet formation, but if π_η<< 1, the droplet size correlates with solution viscosity and also, a broader droplet size distribution will result [41]. The surface tension of liquid formulation also has considerable effect on atomisation; if the surface tension is increased, an increase in electric field is then essential to attain the desirable EHDA mode(s). The necessary applied voltage for stable cone-jet mode will increase with the liquid surface tension as shown in the work conducted by Smith (1986). Also, the atomisation of a liquid is difficult if its surface tension is above ~50 mNm^{-1} (Smith, 1986; Kaiser, Kaiser and Weeks, 1993; David, 1986). However, other studies have indicated that droplet size is independent of the surface tension of liquid.

The relative permittivity is the ability of a material to be polarised in response to an applied electric field. Polarisation reduces the electric field within a liquid. Therefore, the relative permittivity will resist the formation of an electric field within a liquid and further affect the time taken for the charge to reach the liquid cone. Relative permittivity along with the vacuum permittivity and conductivity determines the electrical relaxation time, which is the time needed to level perturbation in the electric charge.

The most important material/physical property for electrospraying in cone-jet (stable) mode is the liquid (or formulation) electrical conductivity. In contrast, if the electrical conductivity is too high, EHDA processing will be impossible due to coronal discharge which impacts the stable cone-jet mode. Liquids which have low electrical conductivities (insulators) cannot be subjected to EHDA processing (e.g. olive oil), even if they can be electrosprayed in cone-jet mode through superficially increasing their conductivities with additives or excipients such as ethanol [43]. According to experimental results obtained by Smith (1986), the lowest limitation of conductivity is 10^{-11} Sm^{-1}. Furthermore, it was found that conductivity had a distinct effect on the morphology of liquid deformation at the nozzle exit during the EHDA process. The jet diameter, jet length, droplet size and flow rate in cone-jet mode all decreased distinctly as the electrical conductivity was increased.

The density of the liquid plays a role in determining the jet diameter in the cone-jet mode. Once the viscosity and electrical conductivity of the liquid formulation are sufficient, the electrical charge is efficiently transmitted across the jet section by viscous forces. However, the viscous force depends on the density of the liquid. At the cone base, the liquid density is of some

importance due to the influence of gravity on the cone shape, although the acceleration of the liquid does not play an important role and is very small. For large capillaries (>1mm), gravitational forces influence the shape of the cone.

1.17 EHDA Spraying (Electrospraying)

Electrospraying is emerging as a widely acceptable method for forming polymeric micro- and nano-scaled structures *via* liquid atomisation. The diameter of resulting electrosprayed droplets range from hundreds of microns down to tens of nanometres. The size distribution of droplets can be near mono-disperse. The generation of droplets and their size can be varied by manipulating the flow rate of the liquid formulation and the applied voltage at the conductive capillary needle. The ejected droplets are electrically charged and this facilitates their motion or deposition on to desired substrates. Electrospraying has been utilised extensively for the encapsulation of therapeutic agents particularly within biodegradable and biocompatible polymeric particles (on the nanometre and micrometre scale. The concepts have also been applied to other existing microstructures which have potentials as dosage forms. For production of near uniform sized particles, the cone-jet mode is the most appropriate and popular EHDA jetting mode. In addition to permitting facile particle size control during process engineering, the ambient nature of the electrospraying technique also limits damage to sensitive pharmaceutical agents. For example, Gulfam et al. (2012) have electrosprayed nanoparticles from natural occurring gliadin polymer for the controlled release of anticancer drug cyclophosphamide; with particles displaying average diameters of 218.66 ± 5.1 nm, demonstrating a narrow size distribution and gradual drug release over 48 hrs. Furthermore, by incorporating gelatine into the existing polymeric matrix system, the release kinetics of the drug was enhanced by expedited delivery of active. This clearly demonstrated the valuable nature of matrix material composition and facile engineering *via* the electrospray process to develop tailor made controlled release nano products as chemotherapies. Cyclophosphamide is a cyclic phosphamide ester of mechlorethamine, which is used in the treatment of neuroblastoma, breast cancer, retinoblastoma and lung cancer *via*cross-linking with DNA which prevents cell division. The electrospraying of silk sericin/alginate systems has also been demonstrated (264-284 µm) but in this instance was focused on the delivery of silk sericin, which in recent times has demonstrated interesting biological properties such as an antioxidant and as a bio-adhesive material. The encapsulation of the active was above 80% and was

released in controlled fashion. BSA, a model protein, has also been used to coat, *via* electrospraying, comparatively coarser lactose particles which are used in numerous pharmaceutical applications. Unlike conventional 'spray' type coatings which spread during coalescence, BSA particles (spherical, ~700 nm in diameter size) retained their structure on the surface of lactose when deposited using ethanol as the vehicle. This demonstrated an interesting approach to prepare coated pharmaceutical excipients, where surface coatings are valuable for adhesion, active protection or immediate release. In contrast, BSA-loaded poly (lactide) (PLA) particles have also been prepared using the single needle electrospraying technique (~particle sizes ranged between 0.84 ± 0.18 to 3.95 ± 0.51 µm with yield in the range of 64.3 ± 1.8 to 80.1 ± 2.6 %) where the encapsulation efficiency of model active was controlled by varying the organic/aqueous phase ratio.

1.18 Coaxial EHDA (coaxial electrospraying)

Single-needle and coaxial electrospraying techniques have similar underlying concepts regardless of differences to experimental nozzle set-up and alignment. As aforementioned, in the single needle electrospraying process, a polymer solution is injected through a capillary nozzle after which it is subjected to an external electrical field (by means of high voltage supply-usually up to ~30 kV). For coaxial electrospraying, a processing nozzle with two enveloped needles (inner and outer needles with different sizes) is aligned concentrically for coaxial electrospraying. Using this technique proteins (such as BSA and lysozyme) have been encapsulated into PLGA particles with clearly visible encapsulation providing opportunities for delicate molecules (e.g. secondary structure of the protein was retained demonstrated using CD spectroscopy). The added benefits included sustained release over a 30 days period and the activity of lysozyme was more than comparable to values reported in other studies. Adaptability in the polymer and drug type, improved drug stability (e.g. for core shell encapsulation technologies), high encapsulation efficiency, controlled release and controlled particle size generation (micro to nano) are the main advantages of the coaxial electrospraying process. Although coaxial electrospraying has been utilised to prepare encapsulated and hollow encapsulated nanoparticles, developmental single needle EHDA processes can also be used to generate porous structures - albeit not as robust as the coaxial system. For the coaxial format the use of multi-material concentric flow becomes a valuable option. Polymers N-octyl-O-sulphate chitosan (NOSC) and tristearin were explored for the encapsulation of model peptide

angiotensin II. High encapsulation efficiencies (~92±1.8 %) and small diameters 100 nm-300nm were achieved. During the coaxial encapsulation process, parameters were optimised to determine peptide stability which was confirmed indirectly in the trout model. Furthermore, and interestingly a distinct difference between *in vitro* and *in vivo* release outcomes was observed which was attributed to environmental and physiological factors. Numerous other studies have focused on co-axial technologies for the preparation of layered and coated particle technologies using chitosan, PLGA and collagen for matrix and compartmentalised drug loading on the micron scale.

1.19 EHDA Spinning (Electrospinning)

EHDA spinning is an efficient and well-known technique for polymer fabrication into well-defined fibrous materials. This technique involves electrostatic forces to be engaged for controlled production of fibres which is similar to the process of electrospraying (for particles). Variations arise due to solution (formulation) and intrinsic polymeric (inter and intra molecular) properties. Unlike the spraying process, the liquid jet remains intact under the instabilities encountered and results in fibre morphology (electrospinning). EHDA spinning has rapidly changed fibre making process from a costly, large scale process, to a laboratory bench scale with a low cost. The primary characteristic of nanofibers is the high ratio surface area to mass (Reneker and Yarin, 2008; Yarin, Koombhongse and Reneker, 2001; Reneker et al., 2000). Advantages offered by the electrospraying process (ambient temperature operation, size control and facile parameter control) are directly applicable for the electrospinning process, however higher output rates are obtained during the electrospinning process technique (Gentsch et al., 2010). These methods have been valuable for several drug delivery and emerging therapeutic systems. For example the generation of biomimetic extracellular matrix (ECM) micro- /nano scale fibres (80 nm – 1.5 µm *via* EHDA spinning technique) were found suitable for tissue engineering (Sell et al., 2007), and have also been used for the fabrication of continuous heteroglycan fibres (on the nano scale); which are useful for regenerative medicine or drug delivery (Lee et al., 2009). Bovine serum albumin (BSA) nanofibers (from a globular protein) have been prepared *via* electrospinning without chemical cross linking, which have potential in biomaterial applications such as wound dressings, suturing and wound closure materials due to its biocompatibility, biodegradability and desirable mechanical properties being met (DrorY, ZivT, MakarovV, WolfH, AdmonA, ZussmanE, 2008). As was the case

with co-axial electrospraying the same format can be deployed to generate layered fibres into several material states (e.g. solids, liquids and gases), which provides added benefits of compartmentalised structures (Ahmad Z, Nangrejo M, Edirisinghe M, Stride E, Colombo P, Zhang HB, 2009). Furthermore, functionalised coating of readily available dressings with a superficial layer of synthetic drug loaded fibres was demonstrated using single-needle electrospinning. In this instance, PVP - indomethacin composite fibres (~3-5µm in width, encapsulation efficiencies of 75% w/w) were directly deposited onto commercial dressing systems (Rasekh et al., 2014). This is an extremely valuable approach as electrospun fibres suffer from poor mechanical properties if prepared as thin superficial layers or films. Nguyen used PLA as fibrous polymeric carrier systems for the delivery of curcumin using electrospinning technique for wound healing applications. An increase of curcumin content in the PLA-curcumin composite from 0.125 to 6.25 wt% yielded a decrease in nanofibre diameter (from 971 ± 274 nm to 562 ± 177 nm). Here, *in vivo* wound healing was assessed in a mouse model showing an increase in wound closure by day 7 for PLA-curcumin (87%) when compared with blank PLA nanofibres (58%) (Nguyen, 2013). Moreover, EHDA spinning of cellulose acetate with lysozyme and rectorite has successfully generated nanofibers with potential in several other areas of combinatorial therapeutic delivery (diameters~500 nm).

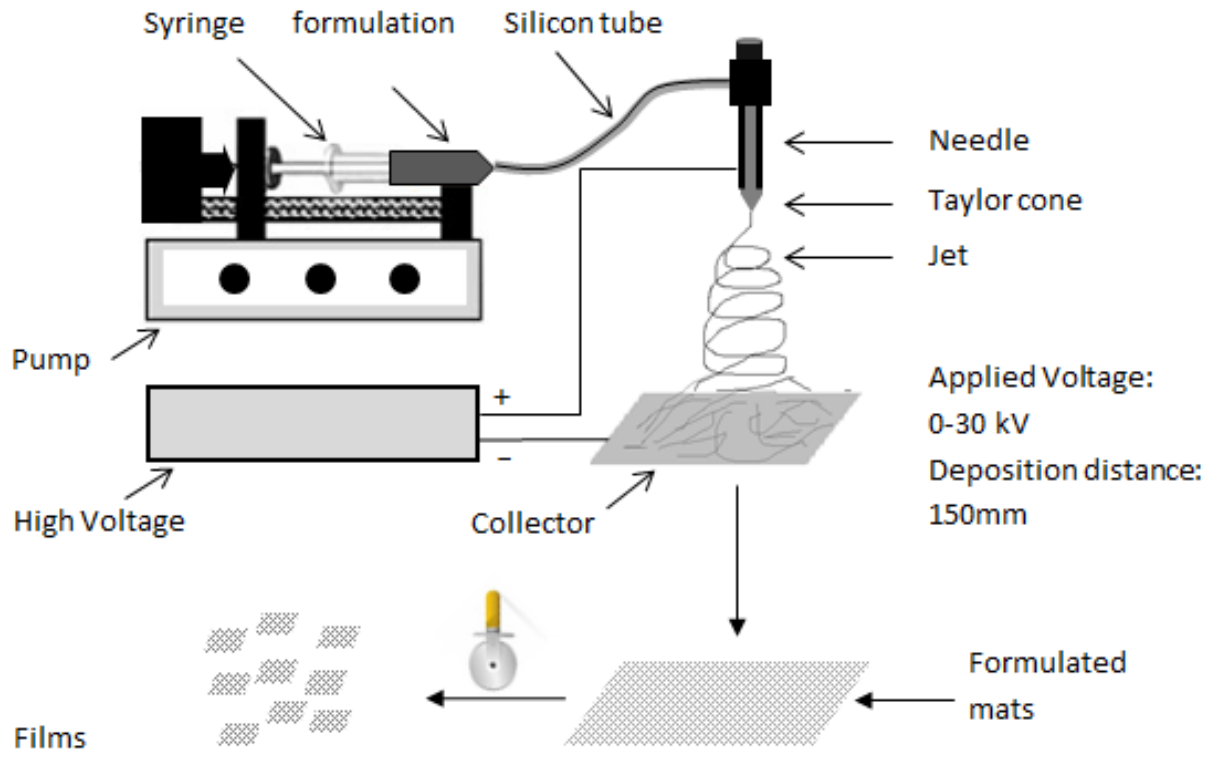


Figure 1. 10 Schematic electrospinning set up

1.20 EHDA Printing

EHDA printing technique is one of the more recent and versatile daughter processes which can be applied to a broad range of materials including polymeric systems. One of the main goals of any printing technique is to enable ordered structure generation based on a pre-determined pathway. The EHDA printing method is valuable as it deploys coarse processing needles/nozzles which are significantly different to many printing techniques where the nozzle exit diameter usually correlates with the pattern size/droplet. The EHDA printing process is electrically driven and operates on identical fundamentals of EHDA technologies (e.g. jet formation), however the printing method has distinct advantages over the electrospinning process, which include controlled patterning and controlled layering of formulation which is valuable for forming with precision. In addition, greater understanding of cell and tissue interaction can be achieved which considers structure and chemistry of polymeric materials. For example, topographic studies (e.g. using living cells) for material coating-cell interactions can be more easily quantified and developed when compared to random electrospun fibres providing mechanisms for spatial and temporal resolution assessment (Wang DZ, Jayasinghe SN, Edirisinghe MJ, 2005). EHDA printing is a computer-controlled method of depositing droplets or threads subject to EHDA in cone-jet mode. The capillary needle is coupled to a high voltage power supply (up to 30 kV). The capillary needles used in EHDA printing can be several hundred micrometres in size, which is about ten times larger than needles used in conventional methods (e.g. ink-jet printing) preventing blockage and allowing easier processing (e.g. forviscous suspensions or rapidly evaporating liquids) as well as generating much finer droplet sizes (Gupta et al., 2007). EHDA printing of polymethylsilsesquioxane (PMSQ, a biocompatible synthetic polymer) solution resulted in complex patterns with printed track widths of ~ 100 µm using a nozzle with an orifice diameter of ~750 µm (inner diameter). Pure polyurethane (PU) was also printed using an overlapped grid pattern (more complex 3D patterning) with a reduced track size of 50 µm (Ahmad, Rasekh and Edirisinghe, 2010). In addition to overprinting, the strength of scaffolds depends on the strut size (print width) which can be varied readily. PCL was used along with bioactive material nano-hydroxyapatite (nHA, ~100nm) to deposit structures of bioactive composite materials which resulted in print widths of 50 µm using an applied voltage range of 8-11 kV, flow rate of 6 µl/min and working distance of 1-2 mm. By adjusting the process parameters; reducing the flow rate to 3 µl/min, printed PCL-nHA structures with 5 µm widths were achieved. The benefits of coarse nozzle diameter utilisation signified the irrelevance of composite clustering (polymer and ceramic nano-

crystals) as pure polymeric PCL structures were patterned to the same resolution. Fibroblast cell behaviour and *in vitro* apatite formation using simulated body fluid demonstrated biocompatibility and cellular/bio-interaction of both types of patterned surfaces (Rasekh et al., 2011; Rasekh et al., 2013). More recently, an electrically driven direct-writing technique was developed which incorporates coaxial principles to deposit layered (encapsulated) micron scaled structures in an ordered or pre-determined pattern using solidified polymers and liquid state excipients such as PEG, olive oil and PMSQ polymer (Ahmad Z, Nangrejo M, Rasekh M, Stride E, Edirisinghe M, 2013).

1.21 Coaxial EHDA Bubbling

Until recently the main objectives of drug delivery were to improve the solubility of drug, increase functionalities of cell targeting and enhance the active bioavailability all in a safe and ethical approach. The competence of conventional preparation methods (e.g. emulsion/solvent evaporation, spray drying) to produce micro- or nanoparticles has evolved significantly in recent times through process enhancement. However, in addition to nanotechnologies, microbubbles have also developed into an area of extreme interest due to several attractive intrinsic properties. These include imaging potential, trigger-based release (e.g. through ultrasound) and several other functionalities arising from the vibrational nature of the thin bubble film coating. Due to the high compressibility nature of the film coat, bubbles serve as effective forms of contrast agents for diagnostic purposes in medical imaging applications. For example, formulated coatings for microbubbles may also propel their utility as active carrier systems, tracing through the body and terminated precisely to release active (using low and high intensity triggers, respectively). This localised approach can be used to reduce side effects of chemotherapies, but size distribution, drug content and destruction are critical; and these are not readily provided by numerous existing bubble preparation techniques. For example, generating microbubbles using sonication or agitation results in wide-ranging size distributions, which means it becomes essential to filter microbubbles to yield samples <10 µm, as greater sizes can cause embolism *in vivo*.

Coaxial EHDA bubbling also utilises similar fundamental principles to those mentioned previously for structure engineering. The difference, however, is the requirement of gas to be infused into the inner needle, which is surrounded by a liquid medium in the outer needle. Typically, in the bubbling process much higher infusion rates (of air) are deployed and new

jetting modes have also been identified due to the interacting nature of multi-phase flow (e.g. coning mode). Using this process (EHDA bubbling) novel hollow spherical structures of silk fibroin have been fabricated which arise from microbubble forming. Upon preparation microbubbles displayed diameters in the range of 240-1000 µm, which can be controlled by adjusting processing parameters as well as the use of combinatorial materials as polymer composites. Furthermore, BSA bubbles have also been developed using the same process and cross-linking to show applications in biosensors (thin porous film membrane engineering), drug delivery (coated BSA bubbles) and tissue engineering (3D porous scaffolds) (Ekemen et al., 2011). Additionally, BSA coated bubbles (*via* bubbling) also record a response to ultrasound demonstrating their potential in both trigger-based release and as potential imaging agents (Ekemen et al., 01). Finally, the preparation of multi-coated bubbles (duplex) using the EHDA process is also possible which paves the way to several opportunities for compartmentalised drug loaded and trigger-based release *via* ultrasound application.

1.22 EHDA Cell Electrospinning

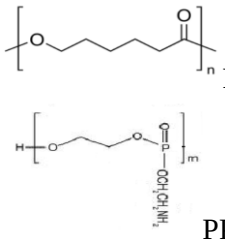
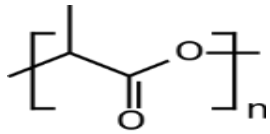
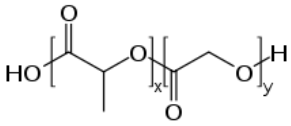
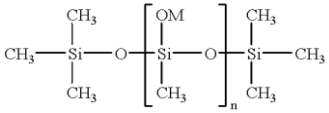
Synthetic biodegradable matrices possessing similar size and mechanical properties of the extracellular matrix (ECM) can be generated using the electrospinning process. As *in vitro* studies and cell infiltration in to pre-prepared electrospun fibres or structures is time consuming and prone to cellular integration challenges, the development of a one-step matrix-cell process is extremely valuable. The electrospraying of smooth muscle cells (SMCs) was demonstrated in tandem with electrospinning of poly (ester urethane) urea (PEUU) yielding a hybrid tissue engineered blood vessel construct in one-step. Hematoxylin and eosin (H&E) staining confirmed qualitatively uniform SMCs. The samples were also tested for their biomechanical properties, which demonstrated appreciable strengths. Furthermore, other studies in this area have utilised both polymeric matrix materials and living cells into one single nozzle formulation which has demonstrated cell viability. These present exciting potentials to develop and replace failing bodily organs which becomes ideal when conventional and novel engineered dosage forms do not respond. On point, the interchangeable nature of EHDA technology demonstrates its value again, this time for therapies we are currently beginning to understand and are yet in their infancy. While these are exhilarating developments, efforts have also been directed towards scaling up simple EHDA processes which will be transferable to

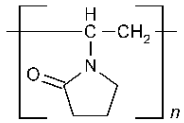
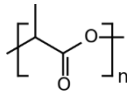
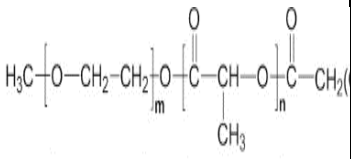
various sub-classes of EHDA processing. These are timely developments which are essential to meet demand once such technologies have matured fully.

Concluding remarks

Synthetic and naturally occurring polymers have been utilised in both well established and emerging pharmaceutical (and therapeutic) materials and dosage forms. The utilisation of these materials has also been expanded from serving as mere fillers and single function matrix materials to controlled release excipients and multi-functional enabling structures. The latter has been expedited due to further functionalisation or careful engineering. EHDA technologies are robust technological platforms permitted the one-step engineering of a selection of polymers (and therefore integration of their unique properties) on several scales which are able to meet requirements from the past and those currently emerging as future therapies including fibres, nanoparticles and bubbles.

Table 1. 2 EHDA of synthetic polymers for use as drug delivery systems

Polymer Type	Polymer (s)	Chemical Structure (s)	EHDA Technique	Name of Drug/ Active	Application	Structure & Dimension	Additional Comments	Ref
Synthetic	PCL-PPE-EA PCL	 PCL PPE-EA	ESy	Bovine serum albumin (BSA)	Therapeutic agent delivery and advanced controlled release	Size range 2-8 µm. Core shell particles	Core shell particles prepared using a single needle ESy technique. Achieved using an emulsion as the liquid formulation. Reduction in burst release.	**53**
	PLA		ESy	Bovine serum albumin (BSA)	Therapeutic agent delivery	0.84 - 3.95 µm. Solid matrix type	Encapsulation efficiency Of BSA was improved by an increase in organic phase ratio. However, these ratios also impact the physical properties o materials	**58**
	PLGA		ESy	Paclitaxel	Therapeutic agent delivery	Down to 250 nm	Particle surface and overall morphology is dependent on polymer concentration.	**54**
	PMSQ		Cox, ESy, ESp, EDHP	Bioactive nanoparticles	Advanced controlled release including use of trigger. Patterning and advanced coatings.	Various structures and sizes prepared (nanometre and micrometre)	Multiple phase encapsulation into micron scaled structures. Controlled release of loaded materials demonstrated using an ultrasound trigger. Layering of structures possible with	**74**

							hollow, solid and liquid phase.	
	PVP		ESp	Indomethacin	Medicated wound dressing and patches	2.58-5.22 µm. Smooth fibres.	Drug loaded fibres dissolve quickly to release active. Direct deposition on to existing dressings provides mechanical robustness to an invisible spun thin coating.	**75**
	PLA		ESp	Curcumin	Medicated Wound-healing patches	971-562 nm Smooth	Increasing the Curcumin content leads to a reduction in spun fibre diameter. Drug loaded PLA fibres demonstrated quicker healing when compared to control blank fibres in mouse models.	**76**
	PEG-PLGA		Cox ESy	BSA and lysozyme	Therapeutic agent delivery and advanced controlled release	800nm–10.4 µm. Various surfaces (smooth to porous).	Drug release sustained for up to 30 days. Bioactivity of lysozyme maintained during the production process (90%) suggesting great potential for other sensitive bio-actives	**59**

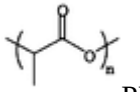
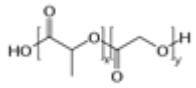
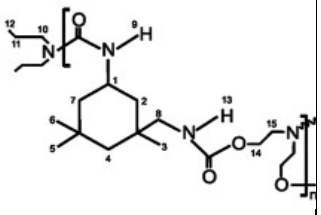
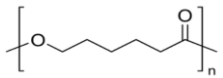
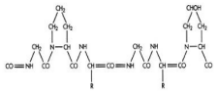
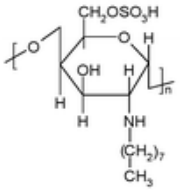
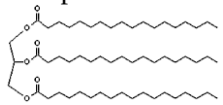
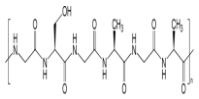
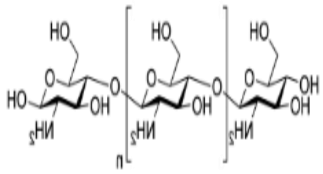
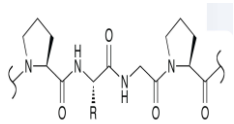
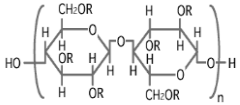
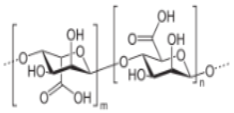
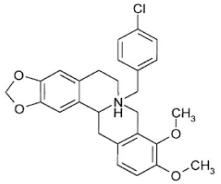
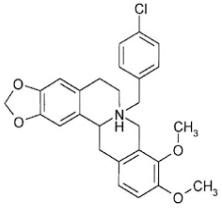
	PLLA-PLGA	 PLLA  PLGA	Cox ESy	Paclitaxel and Suramin	Therapeutic agent delivery and advanced controlled release	15–20 µm	The use of two different types of drugs (hydrophilic and hydrophobic) into a single particle system *via* compartmentalisation is demonstrated. The rate of drug release can be controlled by altering the quantity of inflow for each formulation during Cox ESy	**66**
	PEUU		CellESy and ESp	Living Cells (smooth muscle)	Tissue Engineering and regenerative medicine	Sub micron	Overcoming issues relating to integration and viability of cells into separately engineered matrix systems. The process is one-step	**93**
	PCL		EHDP	Bioactive nanoparticles	Enhanced patterned coatings for improved cellular interaction	Spots, relics and tracks. Tracks optimised to ~5µm	Active hydroxyapatite nano particles embedded into printed PCL matrix tracks demonstrate *in vitro* bioactivity (apatite layer formation in SBF). Structures morphology also variable based on formulation solution concentration (PCL).	**81**

Table 1. 3 EHDA of natural polymers for use as drug delivery systems

Polymer Type	Polymer (s)	Chemical Structure (s)	EHDA Technique	Name of Drug/ Active	Application	Structure and Dimension	Additional Comments	Ref
	Gliadin, gliadin/gelatin	 Gelatine	ESy	Cyclophosphamide	Cancer therapy and controlled drug delivery	Mean size between 218-450 nm	Gliadin based particle systems released drug over a 24-hour period. However, the rate of release can became expedited by incorporating gelatin into the system. Cultured breast cancer cells became apoptotic (24h)	**55**
	N-octyl-O-sulphate chitosan and tristearin	 N-Octyl-O-sulphate chitosan  Tristearin	Cox ESy, ESy	Angiotensin II	Delivery of water-soluble peptides and proteins. Controlled release.	100-300 nm	Peptide stability during the ESy process was demonstrated. A viable processing range (e.g. for applied voltage) was obtained enabling peptide to remain intact. Release of peptide was gradual, and the burst phase was reduced. Interestingly, *in vivo* model (trout) demonstrated a quick on-set of action.	**64**
	Silk, BSA and PEO		Cox BBg	-	Tissue engineering applications and regenerative medicine	240-1000 µm	Greater control on pore size and distribution using this process-ideal for biomaterial applications. Furthermore, as the BBg process requires high inflow rates of gas, this has shown to impact on β-sheet packing within the microstructure. This in turn impacts on mechanical properties of resulting scaffold type materials (e.g Tensile)	**89**

Natural	Chitosan		ESy	Ampicillin	Drug delivery and controlled release. Potential in nasal and GI tract delivery	Mean size ~520 nm. Solid spherical particles	Particles demonstrated encapsulation efficiencies of ~80.4%. Particles also demonstrated clear inhibition zones when cultured on *E. Coli*. Interesting feature to note was the applied voltage rate of 28 kV.	**65**
	Collagen	 R=neutral side chain	Cox ESy and ESy	Theophylline	Pulmonary drug delivery and controlled release	693nm -3 µm	Distinct differences in size and surface morphology was observed when particles were prepared using ESy and Cox ESy. The process required an addition of salt to permit particle preparation for acetic acid based vehicles.	**67**
	Cellulose acetate		ESp and ESy	Lysozyme & Rectorite	Controlled release and medicated wound dressings and patch systems	Mean diameter range ~ 375-580 nm. Smooth fibres	This approach deployed a two step strategy using both ESy and ESp. Cellulose fibres (-ve charge) spun first and then coated with sprayed active (+ve charge) to yield a fibrous composite coated material. The concentrations Lysozymne impacts fibre structure. Samples demonstrated excellent antimicrobial properties against both gram +ve and –ve models (*E. coli* and *S. Aureus*). Release rates were determined over a 2 hour period after which the activity of Lysozyme is lost (in distilled water).	**77**

	Alginate		ESy	Silk Sericin	Protein delivery with potential applications as antioxidant and physical property enhancer	Particle range 264-284 µm. Some variation in particle shape.	Relatively high encapsulation efficiencies were achieved when the ESy approach deployed (>80%). Evidence of release kinetic control.	**56**
	BSA		ESy	-	Advanced particle coatings (e.g. pulmonary delivery)	~700 nm, spherical	A novel approach to coat one excipient (lactose) with another (BSA) but in this instance the coating material retains the sprayed particle geometry (for ethanol based formulations). Due to ambient process conditions; protein particles were stable.	**57**
	BSA		Cox BBg	-	Drug delivery, bio-sensing and regenerative medicine	Multiple BSA formats (porous films, scaffolds and standalone bubbles)	Structure generation controlled by varying BSA solution parameters (concentration) and cross linking of BBg generated structures. Engineered systems demonstrate biocompatibility when tested on mouse cell lines. Improved biosensor properties achieved when compared to traditional membrane (prepared using established method)	**90**

1.23 Quality by design (QbD) framework

Quality by design (QbD) in pharmaceutical development is a concept which is defined as systematic approach to development that starts with predefined objectives and emphasises product and process understanding and process control, based on quality risk management (Buttini et al., 2018; Politis and Rekkas, 2011; Ko et al., 2018). The international conference on harmonisation (ICH) on QbD insist on process understanding and knowledge management. ICH Q8, ICH Q9, ICH Q10 and ICH Q11 are the guideline on QbD. These mudlines direct to structuring of the risk based approaches across the product life cycle and addresses quality of the systems used to formulate the pharmaceutical products, (Bastogne, 2017; Rathore and Winkle, 2009) and moreover provides a framework to support the assurance of product quality as well as continues improvement over the product life cycle (Dispas et al., 2018; Csóka, Pallagi and Paál, 2018; Thakur, Kaur and Sharma, 2017; Ko et al., 2018; Bastogne, 2017).
QbD following three principles which includes first, to assure product efficacy need to identify target product profile (TPP) and critical quality attributes (CQA), second, a risk analysis on drug substances, potential excipients and the definition of design space, third, design a manufacturing process to ensure the reproducibility of the process (H. Wu and Khan, 2009; Stults et al., 2015; Rahman, Siddiqui and Khan, 2013).

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Chapter 2 Methods and materials

This chapter summarises the materials and methods used in this development. Materials will be presented according to the information provided by the manufacturers. Methods are presented as a general protocol in this chapter and will be discussed more in corresponding chapter.

2.1 Materials

Following materials were used to carry out the experiments.

2.1.1 Polymers used in this study

Polyvinylpyrrolidone (PVP), Mw 1300000, used as binder in pharmaceutical tablets.

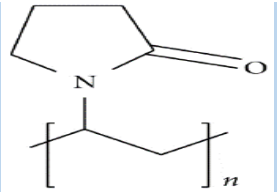
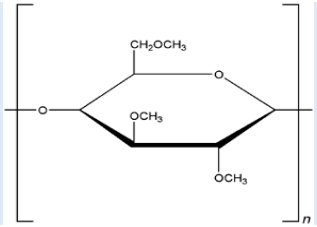
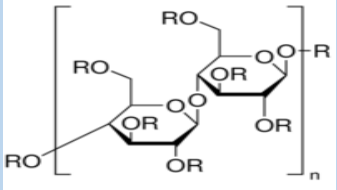
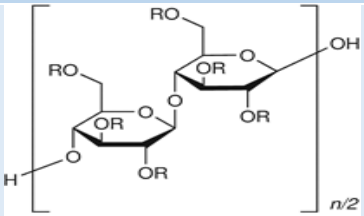
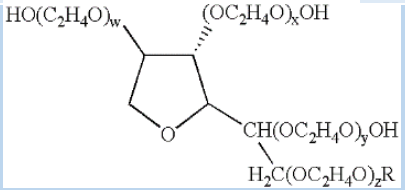
Methocel is a water-soluble polymer which derived from cellulose. Methocel products are used as thickeners, binder and water retention agents

Ethocel is water insoluble, organic soluble polymers which used in many pharmaceutical products as binders, film formers and time release agents.

hydroxypropyl methylcellulose (HPMC) Mw 1261.45 is a semisynthetic viscoelastic polymer which is used in oral pharmaceutical medication as control release and ophthalmic lubricant which is available in many commercial products

Tween 80 is a viscos and synthetic compound which is used as emulsifier and surfactant in food and cosmetic products.

Table 2. 1 Polymer with their chemical formula and functions

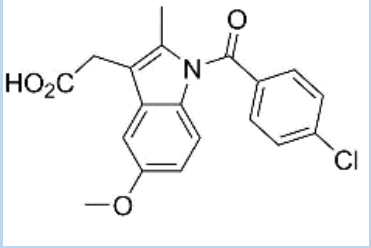
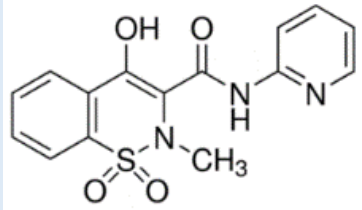
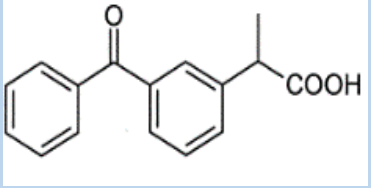
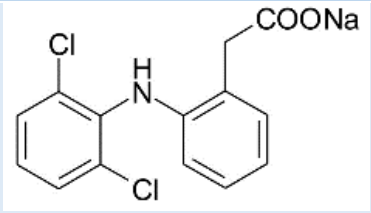
Compound	Chemical formula	Mulocular structure	Function
PVP	(C6H9NO)n		Binder, coating or film forming, release agent to control drug duration of action time.
Methocel	$CH_2CH(OH)CH_3$		Binder, control release
Ethocel	$C_6H_7O_2(OR_1)(OR_2)$		Binder, coating or film forming, control release
Hydroxypropyl methylcellulose (HPMC)	$C_{56}H_{108}O_{30}$		Control drug release
Tween 80	$C_{32}H_{60}O_{10}$		Solubilizing agent (surfactant)

2.1.2 Anti-inflammatory drugs (NSAID) used

These NSAID drugs are potent analgesic. Due to their use for many treatments, they have been prepared in verity of formulations including tablets, capsules, injections, drops, gels, suppositories and ointment. The extensive worldwide use of these drugs has driven researchers to develop and evaluate new methods using numerous analytical techniques.

NSAID (Indomethacin, **Piroxicam**, **Ketoprofen**, and **Diclofenac sodium)**, and PVP (1.3×10^6 g/mol), were purchased from Sigma-Aldrich (Gillingham, UK). Ethanol (HPLC grade) was obtained from Fisher Scientific (Loughborough, UK). Acetonitrile (HPLC grade) was obtained from Sigma-Aldrich, Germany. Ethocel E-10, HPMC and surfactant Tween® 80 were purchased from DOW Chemical Company-UK. Buffer pH 6.8 solution simulating saliva was prepared using NaCl (0.850 g), Na_2HPO_4 (0.200 g) and NaH_2PO_4 $2H_2O$ (0.129 g) in 100 mL of distilled water. All chemicals and solvents were of analytical grade and all components of buffer solutions were purchased from Merck (Germany).

Table 2. 2 NSAID drug and their chemical formula and functions

Compound	Chemical formula	Molecular structure	Function
Indomethacin	$C_{19}H_{16}ClNO_4$		Nonsteroidal anti-inflammatory drug
Piroxicam	$C_{15}H_{13}N_3O_4S$		Nonsteroidal anti-inflammatory drug
Ketoprofen	$C_{16}H_{14}O_3$		Nonsteroidal anti-inflammatory drug
Diclofenac sodium	$C_{14}H_{10}Cl_2NNaO_2$		Nonsteroidal anti-inflammatory drug

2.2 Methods

2.2.1 Electrospinning formulations

Electrospinning is a one step process in which the formulation solution atomised by a nozzle. Formulation compositions are listed in a Tables for each chapter. Polymer solutions (100 mL) were prepared using a base of ethanol and distilled water (80/20 v/v%). PVP (5% w/v) was then dissolved in the vehicle through magnetic stirring (Jenway, Staffordshire, UK). Selected co-polymers (2 and 5% w/w of PVP) and NSAID (5% w/w of PVP) were added to each formulation under stirring (at ambient conditions) until complete dissolution. Paraffin film was used to seal resulting solutions to prevent solvent evaporation. Finally, four additional formulations were prepared using selected solutions of PVP, co-polymers (at 5% w/w) and NSAID but with addition of 5%w/w Tween® 80 (of PVP). All solutions were prepared at the ambient temperature. The conventional pharmaceutical function of excipients used in this study is shown in Table 2. 1.

2.2.2 Solution characterization

2.2.2.1 Electrical conductivity

Most of solution conduct electricity to some extent. The ability of solution to conduct electricity can be measured by using conductive meter. Different materials have different effect on conductivity of formulation. For example, adding salt will increase conductivity of formulation. The current flows by ion transport. The more ions presented in the solution the more conductive the solution will be otherwise the solution will be resistive to current flow. Conductivity is measured by immersing the sensor into the solution. Using Ohm law; the current pass through between electrodes is proportional to the current across the two electrodes.

$$I = \frac{V}{R}$$ Equation 2. 1

Where I (units of amperes) is current through the conductor, V (units of volts) is the voltage measured and R (units of ohm) is the resistance of the conductor.

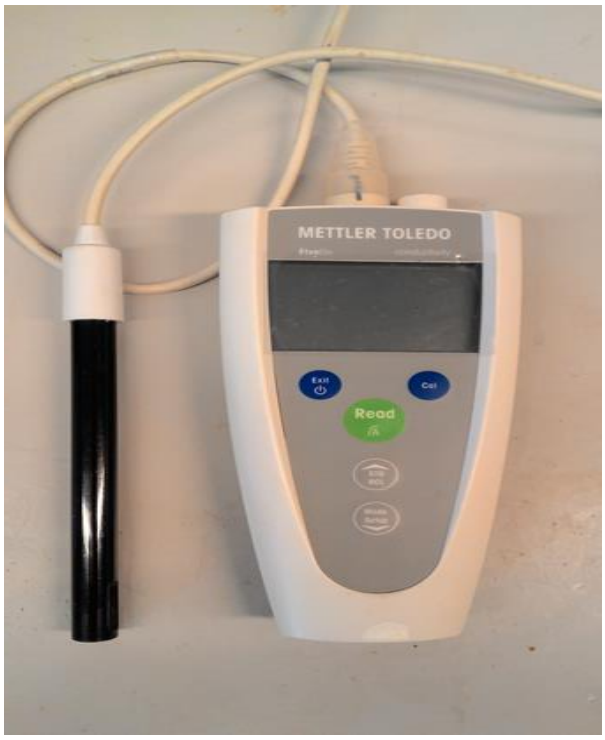


Figure 2. 1 Digital image of a Mettler Toledo Electrical conductivity meter

2.2.2.2 Viscosity

One of the properties of liquid is its resistance to flow, this can therefore be measured by a viscometer. Since changes in viscosity reflect changes to molecular weight, viscometer is used to characterise the formulations. Viscosity of the formulation (drug and polymer) was determined by using a sine wave vibra viscometer (mPa.s). Change in viscosity on real time basis can be detected by thin and small size sensor plate. The electric current which is needed to vibrate the two sensor plates at constant frequency of 30Hz is measured as viscosity. This is useful technique in determining the ideal polymer or combination of polymers for electrospray or electrospinning as flow rate and viscosity are main aspect to be considered for this development.

Figure 2. 2 Digital image of Sine-wave vibra viscometer (SV-10)

2.2.2.3 Surface tension

Du Noüy ring method was employed to measure surface tension of formulations. The force which acting on a ring (wetted length) because of the tension of moving ring from one phase (solution) to another is measured. This is done by inserting the ring into solution at the surface of solution. When the ring is forced out of the surface, the required force is the surface tension of that solution and can be measured using the following equation:

Equation 2. 2

$$\sigma = \frac{F}{L \cdot \cos\theta}$$

Where σ is surface tension, F is the force, and L is the wetting length of the ring

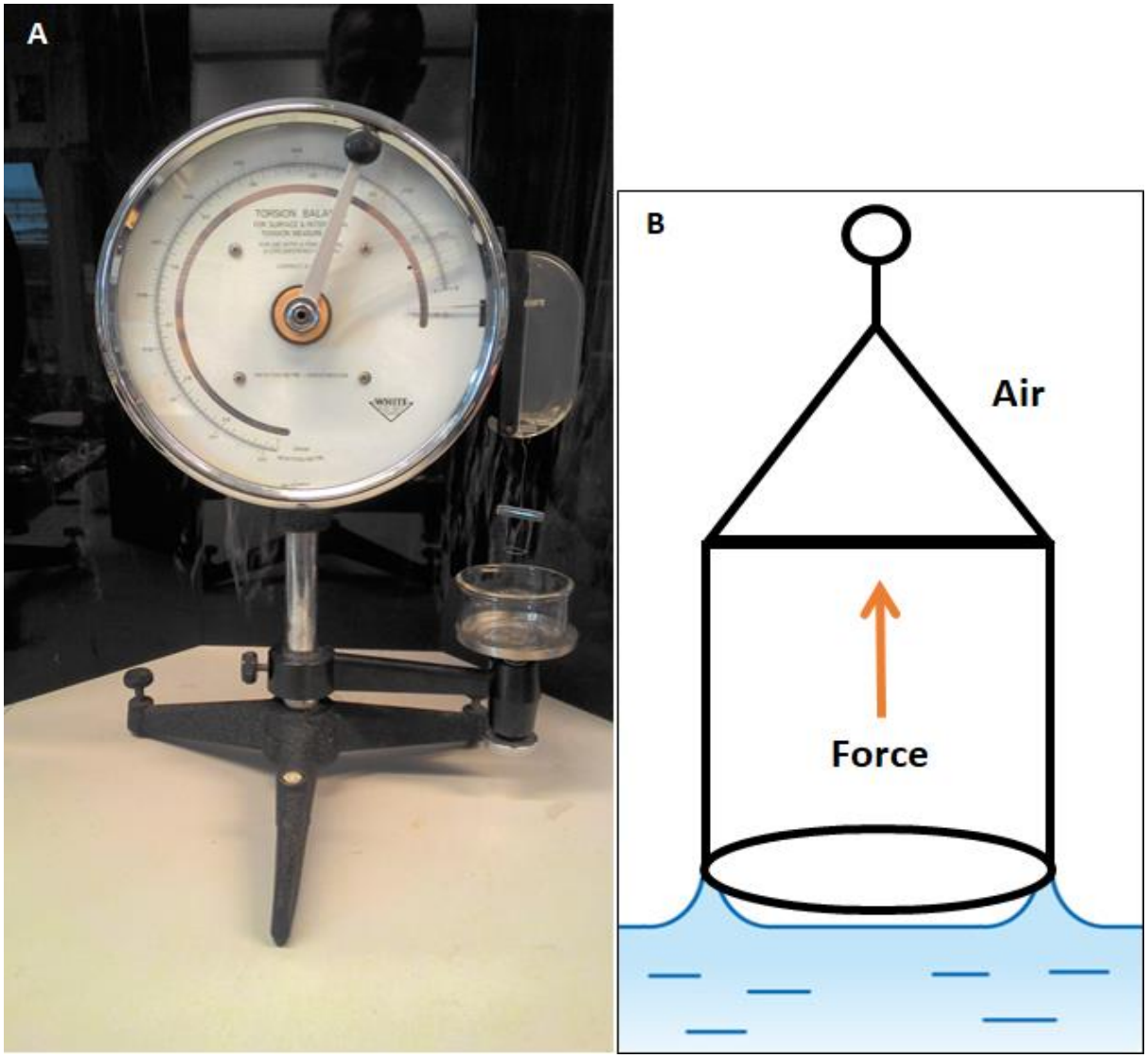


Figure 2. 3 Digital image of Torsion balance (A) and Schematic diagram of the Du Noüy ring method (B)

2.2.2 Electrospinning

The basic arrangement for electrostatic atomisation is, a nozzle connected to the high voltage power supply and supplied with a liquid (formulation) to be atomised. As the solution is stretched with electrostatic repulsion between the surface and the evaporation solvent the droplet changes its shape to conical and, if the voltage is high enough, a liquid filament is ejected from a cone peak.

During electrospinning the liquid is flowing out of the nozzle and is forced by an electrical field to be dispersed into fine fibres. Controlling of parameters such as the flow rate of the liquid and the voltage give the chance of formation of controlled fibre size.

Electrospinning technique involves the use of a high voltage to induce the formation of a fibre (micro or nano scale) from a liquid. As the viscous polymer solution is stretched with electrostatic repulsion between the surface and the evaporation solvent a solid fibre will be generated. In electrospinning process parameters such as electric field, diameter of needle, flow rate of solution, etc. and the property of solution such as viscosity, concentration surface tension, etc. have a direct effect on the morphology and diameters of fibres Electrospinning can prepare particularly fine fibers from a polymer solution by utilizing a simple one step, electrostatic interactions and elongation of the viscoelastic jet system process.

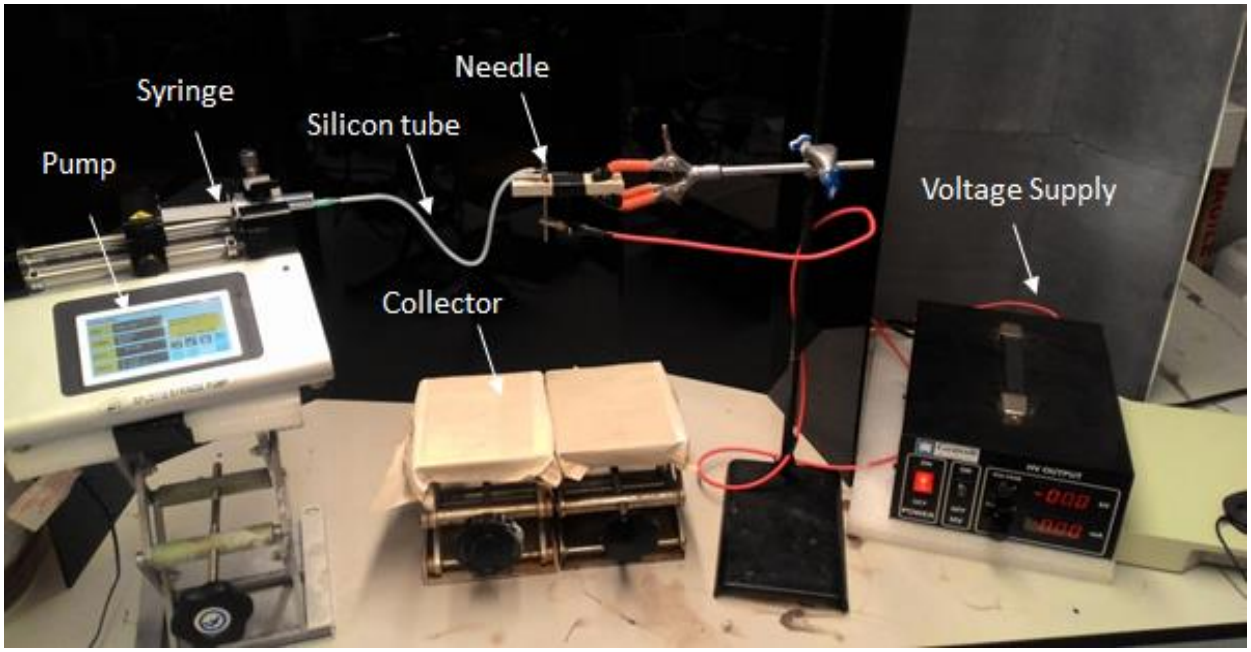


Figure 2. 4 Electrospinning equipment's (Pump, syringe, needle, collector, and voltage supply)

2.2.3 Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) is one of the thermal analytical techniques which allows for direct study of thermal stability of drugs (Spink, 2008). The principle of DSC is that it monitors heat flow between a sample and an inert reference sample during a specified temperature (Garbett et al., 2009). In DSC, a sample and a reference are heated or cooled at the same rate (Farah et al., 2018). The heat flow difference required to maintain zero temperature between the sample and the reference is measured as function of time and temperature (Knopp et al., 2016; Johnson, 2013). The purpose is to deliver an assessment of the activation energy at the glass transition temperature, as well as of the fragility index, of amorphous NSAID from

DSC data. Upon heating, the fibres undergo some thermal induced event such as; endothermic or exothermic reaction. These reaction causes changes in the differential heat flow which is then recorded as a peak on the DSC thermogram (Clas, Dalton and Hancock, 1999). The area under peak is directly proportional to the enthalpic change and its direction indicates whether the thermal event is endothermic or exothermic (Senatra, 2006). The endothermic reaction, in which heat flows into the sample, the system absorb energy from surrounding usually in the form of heat. However, exothermic reactions in which energy flows out of the samples in the form of heat. DSC helps to study the transitions such as apparent denaturation melting temperature (T_m), crystallization, and glass transition temperature of NSAID formulations.

DSC methods has the advantage of being a relatively fast technique with most analysis taking less than thirty minutes for each sample. Different type of materials can be analyzed by DSC e.g. solid, liquid, films and powder.

DSC was performed using a PerkinElmer Jade DSC differential scanning calorimeter (PerkinElmer Ltd., Shelton, CT, USA). The heating flow rate was calibrated with pure indium standard. Samples (3.5–4.2 mg) were placed into an aluminum pan (Perkin Elmer) and sealed. Samples were heated from 20 to 200 °C at a heating rate of 10 °C/min under a nitrogen purge of 70 mL/min.

Figure 2. 5 Digital image of Differential Scanning Calorimetry (DSC)

2.2.4 Thermal Gravimetric Analysis

Thermal gravimetric analysis (TGA) is another thermos analysis technique like DSC. TGA provides very useful and relevant information for materials of interest. TGA measures and record melting point, transition temperature and weight loss of materials (Bach and Chen, 2017). TGA measures the changes in a sample as a function of temperature or time. Changes will appear in chemical or physical properties of samples and therefore weight loss. Thermal stability of sample can be achieved by two methodologies; fixing the temperature over a range of time or by ramping the temperature over a fixed time.

Thermal behavior of fibrous films was analyzed using a thermal gravimetric analyzer TGA (Perkin Elmer Pyris 1 TGA). Each sample was placed in an aluminum pan (TGA, Perkin Elmer), weighted (7–10 mg) and heated using a temperature range of 20 to 800 °C in nitrogen atmosphere, using heating rate of 10 °C/min.

Figure 2. 6 Digital image of Thermal gravimetric analysis (TGA)

2.2.5 Fourier Transform Infrared Spectroscopy (FTIR)

Infrared spectroscopy is divided into three segments, the near-IR, mid-IR and far-IR. Fourier transform infrared spectroscopy (FTIR) is an analytical technique used to study the interactions between infrared light with matter. FTIR is a common analytical technique that is popular and used vastly in pharmaceutical sample identification. FTIR is a non-distractive, fast and quotative technique which measures the absorption of infrared radiation by the sample material versus wavelength in line or off line (Großhans et al., 2018; Alvarez-Ordóñez et al., 2011). A spectrum is produced representing the component of the sample in relation to their functional group and bonds in term of peak which also have a corresponding wave number and therefore, identify molecular compound and structures (Kamnev et al., 2018). Upon radiation of a material by infrared, molecules are excited into higher vibration state. The result wavelengths of sample which identify the functional groups present are characteristic of its molecular structure (Hou et al., 2018).

The chemical structure of raw materials and formulated fibrous films was analyzed by Fourier transform infrared spectroscopy (FTIR Platinum-ATR fitted with Bruker Alpha Opus 27 FT-IR). Infrared spectra were recorded in the range 4000–400 cm^{-1} using 30 scans at a resolution of 4 cm^{-1} for each sample.

Figure 2. 7 Digital image of Fourier Transform Infrared Spectroscopy (FTIR)

2.2.6 Raman spectroscopy

Identifying the properties of raw materials and excipients are important in development of pharmaceutical products. On the other hand, helps to regulate the critical process parameters and quality attributes (Paudel, Raijada and Rantanen, 2015). Raman spectroscopy was employed as feasible application to PAT to investigate intermolecular vibration, rotational and other low frequency of formulations in the solid state. In Raman spectroscopy laser interact with the system and resulting in shift into the energy of laser photons (Doty and Lednev, 2018). The shift gives information about molecular bonds and the molecule itself (Kong et al., 2015). Therefore, provides the product chemical and structural information. Raman spectroscopy is a nondestructive, fast, and easy technique and facilitate quantitative and qualitative analysis (Maurer et al., 2018; Bumbrah and Sharma, 2016).

Raman spectra were collected using a FORAM® Raman spectrometer (Foster & Freeman Ltd) equipped with a 785 nm laser, using 30 scans at a resolution of 4 cm^{-1}. Raman spectra were collected between 3300 and 100 cm^{-1}.

Figure 2. 8 Digital image of FORAM® Raman spectrometer (Foster & Freeman Ltd)

2.2.7 Scanning Electron Microscope (SEM)

Scanning electron microscopy (SEM) is a widely used image analysis in many scientific applications. SEM operates at high vacuum. Emission gun generate beam of electron and then the beam of electron pass through electromagnetic lenses and therefore, produce thin beam of electrons (Lewis, 1992). Thereafter, the beams scan the surface of a sample with a focused beam of electron and thereafter, electrons emitted from the surface to be collected by detector (Klang et al., 2012; Bogner et al., 2007; Panessa-Warren, 1983). Interaction of electrons with atoms in the sample yield indications that comprise sample surface topography and composition (Klang, Valenta and Matsko, 2013; Carter and Shieh, 2015; Singh, 2016). SEM with secondary electron detector can visualise surface morphology, shape, and surface of samples. On other hand optimisation of particle size, roughness and morphology of

pharmaceutical materials can be achieved. Furthermore, these aspects are the key crucial in compounding process and product solubility pharmaceutical products (in this study fibres) either comprising active pharmaceutical ingredients (API) or excipients.

SEM was employed to analyses the morphology of the fibres. It is also can be used as identification tool, as each material has a unique and different effect on fibres structure as well as their morphology.

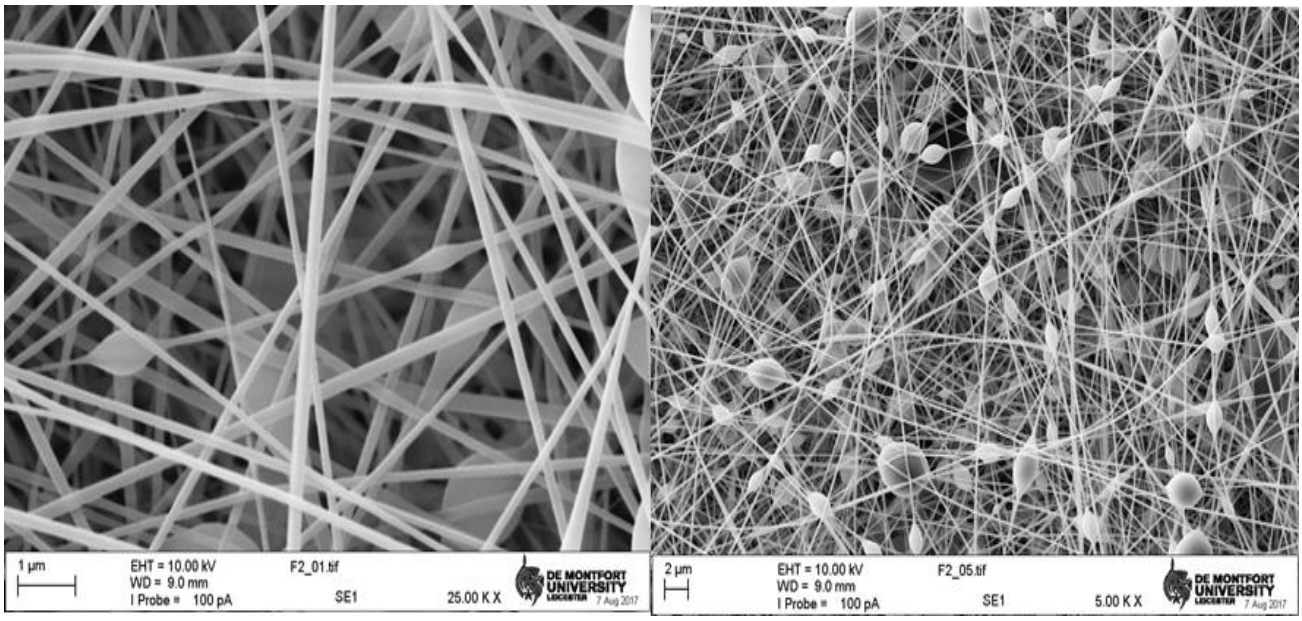


Figure 2. 9 Scanning electron microscopy of fibres at 5000 and 25000 magnifications.

Scanning electron microscopy (SEM) was conducted using SEM Carl Zeiss EVO® HD15 (Carl, Zeiss, Oberkochen, Germany) at an accelerating voltage of 5 kV. Fibrous membranes were mounted onto a sample holder (aluminum stub) using double sided tape. To prevent any overcharging, samples were sputter coated (S150B, Edwards, Crawley, West Sussex, UK) by exposing samples to a gold atmosphere at 5 Pa. Each sample was coated for 105 s twice. Fibre diameters were assessed *via* Image Tool program, and fibre diameter distribution was determined using a random sample size of n = 100 fibres.

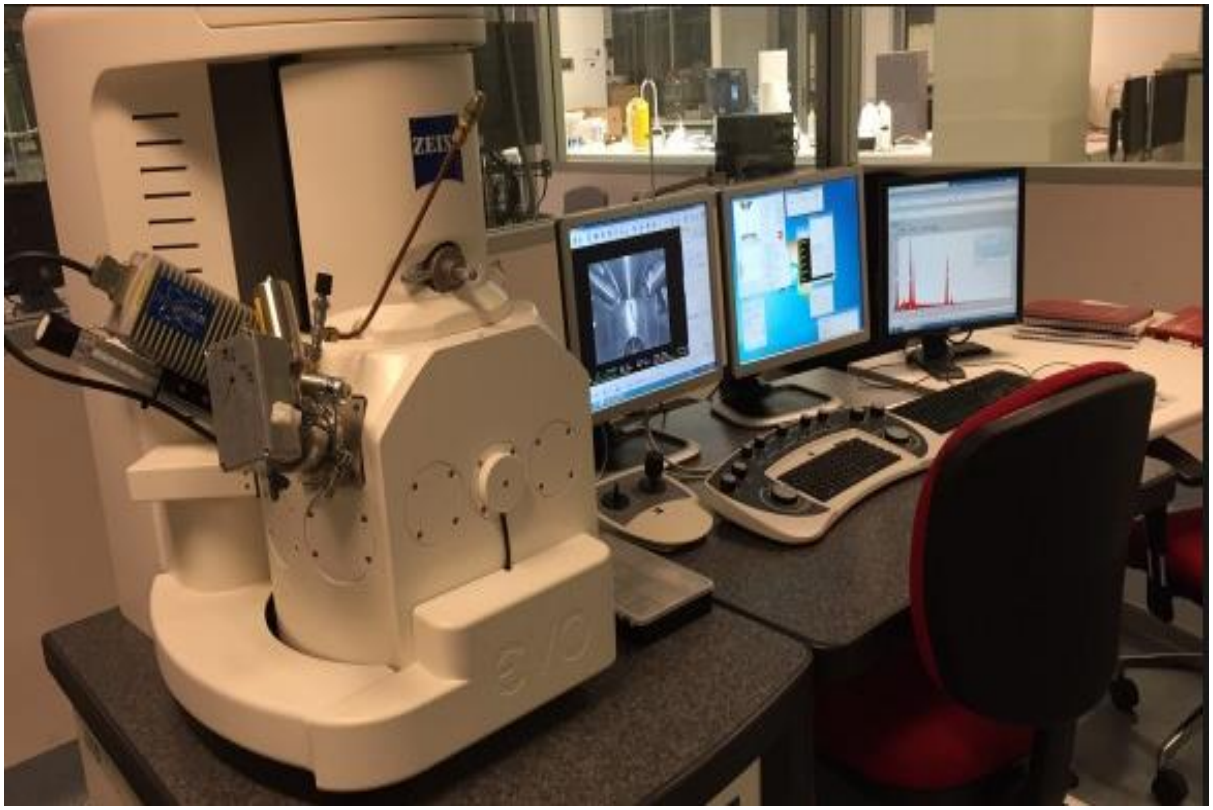


Figure 2. 10 Digital image of scanning electron microscopy (Carl Zeiss EVO® HD15)

2.2.8 Atomic force microscopy (AFM)

Characterisation of small specimens become easy with the help of microscopes. Microscopes uses photons and electrons (probes) in optical and electron microscopies, respectively. Atomic force microscopy (AFM) is a high-resolution scanning probe microscopy which look at samples on the atomic level. This technology is common used in nanotechnology. AFM uses the impact of the sample force on the probe to form a three-dimensional image of sample surface (Wang and Nie, 2018; de Pablo, 2018).

AFM does not use laser or irradiation, and this is its advantage upon other technologies (e.g. electron microscopy and optical microscopy). Therefore, does not need to create a vacuum to guide the beam or staining (metal coating) the sample (Chang et al., 2012; Liu and Yang, 2018).

Three dimensional analysis was also performed using atomic force microscopy (AFM) [AFM 5420 (Agilent Technology, Berkshire, UK)]. AFM was conducted in contact mode using a radius tip of 15 μm and a cantilever spring constant of 0.2 N/m.

2.2.9 X-ray diffraction (XRD)

Atomic structure of materials necessary to understand and predict properties of material under study. XRD has shown that has potential use in studying the structure of materials (Petkov, 2008).

X-ray diffraction (XRD) is a rapid and nondestructive analytical technique which used to in provide information about structural, physical properties and chemical composition of materials in pharmaceutical industry (Brittain, 2003). The technique offers identification and characterization of materials based on their diffraction outline. In this technique an X-ray beam hit the sample and scattered intensity from atom within the target sample observed as wavelength or energy. The cooperation of the incident beams with the sample yields constructive interface. Thereafter, the diffracted beams are detected and processed. Thus, XRD has proven to be a valuable technique in research and pharmaceutical industry for crystalline materials (Petkov, 2008; Rompalski et al., 2016).

Sample crystallinity was evaluated using XRD analysis performed on a Bruker D8-Advance diffractometer, operating at 40 kV and 40 mA whilst using Cu Ka1. A scanning rate of 0.35 s/step was used. Spectra were collected in 2 theta from − 3 to + 160°.

Figure 2. 11 Digital image of **X-ray diffraction (XRD**

2.2.10 Encapsulation efficiency (EE)

Precisely weighted amounts (10 mg) of each fibrous film sample was dissolved in 20 mL of PBS in capped glass vials under magnetic stirring for 15 min to ensure complete dissolution. The resulting solutions were centrifuged at 4500 rpm for 15 min, and quantification of NSAID drugs were performed on the resulting supernatants using UV–Vis analysis at 320, 354, 260 and 276 nm for Indomethacin, Piroxicam, Ketoprofen, and Diclofenac Sodium respectively (UV–Vis spectrophotometer, Shimadzu, UVmini-1240). Experiments were run in triplicate. Drug content was calculated according to**Error! Reference source not found.**.

$$\text{Encapsulation efficiency } \left(\%\frac{\text{w}}{\text{w}}\right) = \left(\frac{\text{Weight of loaded drug}}{\text{Weight of drug in fibre}}\right) \times 100 \qquad \text{Equation 2. 3}$$

Drug encapsulation efficiency is an important index for drug delivery systems. Where encapsulation efficiency is the percentage of drug that is successfully entrapped into the nanofibres.

2.2.11 *In vitro* release studies

Release studies were conducted in capped glass vials in an agitating water bath at 37 °C. 5 mg of every sample was sunk to the bottom of vials filled with 20 mL of PBS buffer solution pH 6.8 (Ladrón De Guevara-Fernández, Ragel and Vallet-Regí, 2003; Karavasili et al., 2014). At predetermined time intervals samples of 1 mL were withdrawn and replaced with equal volumes of fresh and pre-heated to 37 °C buffer solutions. Samples were centrifuged at 2500 rpm for 15 min, and the supernatants were analyzed with UV–Vis at 320, 354, 260 and 276 nm for Indomethacin, Piroxicam, Ketoprofen, and Diclofenac Sodium respectively. All experiments were repeated four times. The calibration curve of al NSAID drugs in PBS pH 6.8 showed good linearity (r^2 = 0.999) in the concentration range 1–40 ppm.

2.2.11.1 Release kinetic modelling

The system that drug get into the body is called drug delivery system. It includes active drug, bio active agents and other excipients which is used as vehicle for administration of the active drug. In recent years drug therapies with polymer-based drug delivery systems has been improved largely. Such a system essentially improves patient compliance and transport drug molecules effectively. To achieve appropriate drug release and proper absorption of active drug certain tests must be performed (e.g. dissolution). Polymer delivery system can be completed with mathematical modelling to control release system. Mathematical modelling can reduce the amount of experiment needed by help to predict the drug release rate and diffusion behaviour from the formulation. Therefore, the development of mathematical models facilitates us with prediction delivery performance. Many mathematical models have been developed to demonstrate different drug delivery system. Various models such as zero order, first order, Higuchi, and Korsmeyer-Peppas can be used to plot in-vitro release profiles.

2.2.11.2 Zero order Kinetic

The rate of drug release is constant with zero order kinetic and the release independent to drug concentration of the dissolved substance.

$$Q = Q_0 + K_0 t \qquad \text{Equation 2. 4}$$

Where Q is the amount of drug release at time t, Q_0 is initial amount of drug, K_0 is the zero order rate constant and t is time at which the amount of drug released.

2.2.11.3 First order Kinetic

The rate of drug release is proportional to the drug concentration with first order. The rate of release increases with the increase in drug concentration.

$$\text{Log } Q_t = \text{Log } Q_0 - Kt/2.303 \qquad \text{Equation 2. 5}$$

Where is Q_t the amount of drug unreleased at time t, Q_0 is initial amount of drug, K is first order release constant, and t is time in hours.

2.2.11.4 Higuchi model

The Higuchi model proposes that the drug release by diffusion and relates drug concentration to the square root of time.

$$Q = K_H t^{1/2} \qquad \text{Equation 2. 6}$$

Where Q is the quantity of drug released at time "t" per unit area, K_H is the Higuchi dissolution constant and t is time in minutes. The cumulative percentage of drug release against square root of time plot gives straight line.

2.2.11.5 Korsmeyer Peppas model

The Korsmeyer-Peppas is implies that the fraction release of drug is exponentially related to release time. Diffusion or non-fickian diffusion refers to combination of both diffusion and erosion rate release.

$$M_t / M = K_m t^n \qquad \text{Equation 2. 7}$$

Where M_t is the amount of drug released, M is the total amount of drug in dosage form, K_m is kenotic constant, n diffusion or release exponent, and t is time in hours. The linear regression of log (M_t / M) against log t plot estimate the n value.

Table 2. 3 Iinterpretation of diffusional release from polymeric films.

Release exponent (n)	Drug transport mechanism	Rate as a function of time
0.5	Fickian diffusion	$t^{-0.5}$
0.45< n = 0.89	Non- Fickian transport	t^{n-1}
0.89	Case II transport	Zero order release
Higher than 0.89	Super case II transport	t^{n-1}

Drug release data was further assessed using the Higuchi model as shown in (**Error! Reference source not found.**). The mathematical model is necessary to study the release mechanism of the prepared formulation. The Higuchi model was selected based on previous studies which indicate diffusive mechanisms for drug entrapped within filamentous matrixes (Kataria et al., 2014; Maleki et al., September 2016; Immich et al., 2013). The Higuchi model specifically describe the release rate of drug from a matrix system. In this system the drug dissolved from surface layer of matrix to the external solution and therefore, the dispersed drug moves into the interior as a forward-facing.

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Chapter 3 Preparation of buccal film with different polymer and polymer-copolymers

3.1 Introduction

Active pharmaceutical ingredients (APIs) can be administrated to the body using various routes; broadly classified as parental or enteral. Selection of a specific route (*e.g.* oral, transdermal, *i.v.*) is driven by the intended purpose, method of action and several patient focused factors (Cook, 2007). The administration route is a major aspect of drug delivery as it is responsible for drug accessibility. Therefore developments yielding novel dosage forms or enabling technologies which overcome limitations associated with conventional systems become essential (Gurruchaga et al., 2015).

Emerging technological platforms within the pharmaceutical arena have provided elaborate designs and techniques to enhance existing formulations and drug administration routes or to accommodate problematic chemical entities (Mehta et al., 2017). The parenteral route is beneficial due to direct active delivery into the bloodstream, however, due to poor patient compliance and potential anaphylaxis; its regular use for conventional drugs is limited. In contrast; pediatric, geriatric or other patient groups with swallowing difficulties (Lopez et al., 2015a) limit the use of traditional oral dosage forms such as tablets, capsules, and liquids.

Such dosage formulations also require in depth assessment for *in vivo* and shelf stability, dosing regimen and sustained release. One other crucial aspect relates to dosage form development. Although unit operations vary between each form, there is also a need to ensure the correct drug content is loaded, and in the case for solid dosage forms, there is limited flexibility for patients with allergies or other inhibiting factors (*e.g.* anatomical or physiological). For these reasons, efforts to improve formulation and dosage form design have multiplied over the last decade (Lopez et al., 2015a).

To this end, various formulations have been developed including fast disintegrating tablets, lozenges, sublingual tablets and buccal films (Khan et al., 2016). The buccal route for drug delivery has generally focused on rapid drug absorption once the dosage form (*e.g.* film or pill) is positioned in the buccal cavity; benefitting from the large volume of sublingual capillaries and their proximity to the mucosal layer. Plus, drugs have been shown to enter systemic

circulation directly *via* the jugular vein (Lopez et al., 2015b; Marxen et al., 2016; Thompson and DiMartini, 1999; Fonseca-Santos and Chorilli, 2018a). The avoidance of first-pass metabolism and intermediate permeation properties makes this an attractive option for pH and enzyme sensitive actives (Marxen et al., 2016).

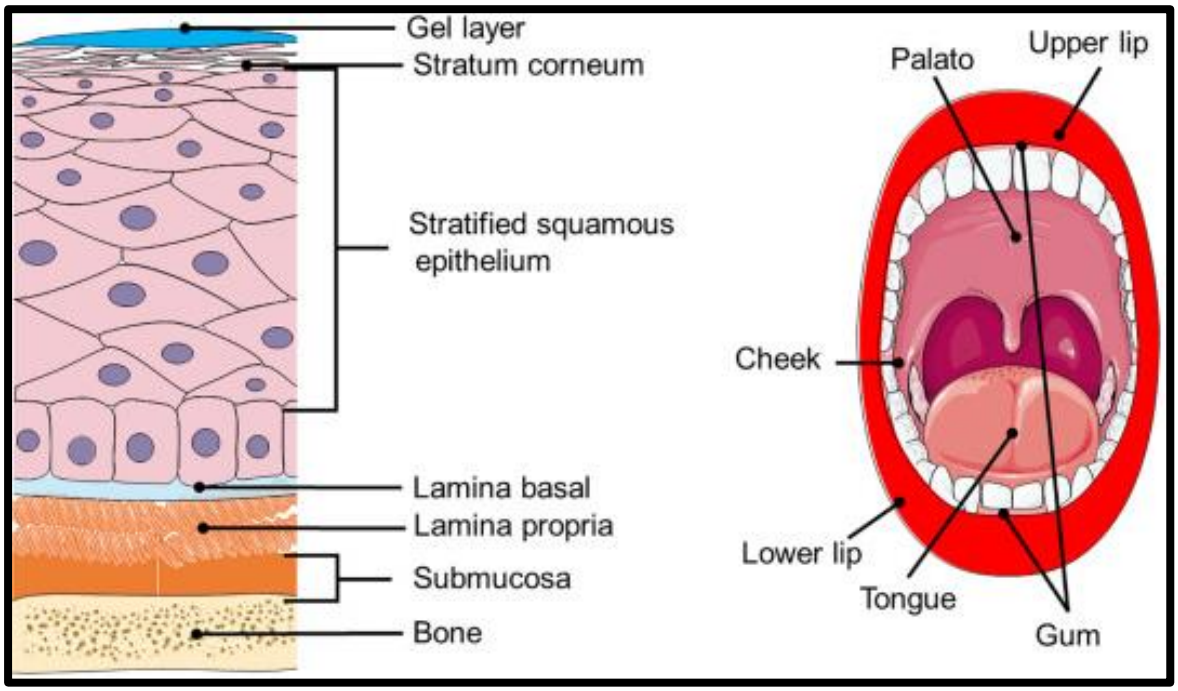


Figure 3. 1 Schematic of oral cavity and buccal mucosa histology representative (Fonseca-Santos and Chorilli, 2018b).

Additionally, the highly vascularized nature of the buccal mucosa offers other added advantages (*e.g.* fast onset of action). Active pharmaceutical ingredients (API's) such as nitroglycerin have been developed into sublingual dosage forms (*e.g.* for the treatment of angina) and several types of mucoadhesive formats for buccal delivery have been developed including gels, ointments, patches, and tablets *e.g.* heparin as a gel system (Radivojša Matanovic et al., 2015). Finally, rapid cellular recovery is another advantage offered by the buccal route (Attia et al., 2004; Salamat-Miller, Chittchang and Johnston, 2005; Boateng and Okeke, 2014; Meng-Lund et al., 2014).

Indomethacin (INDO) belongs to the nonsteroidal anti-inflammatory drug (NSAID) category; which is conventionally used to reduce fever, pain, stiffness and swelling. INDO's mode of

action is to inhibit production of hormones-like substances (prostaglandins) which cause inflammation and pain (Inada et al., 2013). INDO is a hydrophobic drug which is generally available in crystalline form. The importance of amorphization is increasing in consequence of its value in the pharmaceutical industry for several reasons, including the development of poorly soluble active pharmaceutical ingredients and compressibility. Drug amorphization is a viable route to enhance solubility and dissolution at the buccal cavity. This in turn has potential to increase active bioavailability (Alqurshi et al., 2016).

In this study, fibrous films for buccal delivery of INDO were formulated and fabricated using one-step electrospinning (ESp) technique. The ESp process is an efficient method for nanometre and micrometre fibre production using electric fields (~ 15–30 kV) at the ambient environment. During the ESp process formulated drug-polymer solutions are stretched into filamentous structures arising from electrostatic repulsion between the collecting platform and the tip of the formulated media ejecting nozzle (Ahmadipourroudposht et al., 2015). The process is suitable to produce composite drug fibres using large and complex molecules, without detailed coagulation chemistry, elevated temperature or pressure. Several other benefits include facile control on fibre size, shape, and surface charge. Formulation flow rate, applied voltage and deposition distance are widely considered as critical factors for fibre morphology type whilst material selection significantly affects structure production ability (*e.g.* viscosity, electrical conductivity, surface tension and density)(Haj-Ahmad et al., 2015; Mehta et al., 2017). Finally, two further crucial aspects of the ESp process in relation to dosage form preparation is the ability to incorporate crystalline actives in the amorphous state (more dispersed throughout the filamentous structure) and to control drug volume (width) in resulting films through formulation deposition time (Rasekh et al., 2014) providing a route to modulate drug content.

Employing of polymers carriers in modern drug delivery to produce spatiotemporal release of therapeutics starts with site specific delivery products and implanted reservoir products. Appearance of effective and definite biological therapeutics has intensified the motivation for intelligent delivery systems (Liechty et al., 2010).

Polymer science has advanced the development of several novel drug delivery systems. Surface and bulk properties of polymers can help to be designed for numerous drug delivery applications. Among them, biodegradable polymer attracts more interest as they can be degraded into nontoxic monomer within the body (Pillai and Panchagnula, 2001). On the other

hand, blending different polymers with unlike properties could yelled a mixture of striking valuable and reasonable way of gaining new structural materials (Bajpai et al., 2008; Yoo et al., 2000). Polymer blend advantages over controlled release applications possibly will include fabrication of devices, drug loading and enhanced release properties of formulation. Moreover, with blend polymer manipulation of the device properties such as hydration, mechanical strength and degradation rate can easily be controlled (Bajpai et al., 2008).

In the current work, amorphous fibrous INDO films were prepared using conventional pharmaceutical excipients (Polyvinylpyrolidone (PVP), Ethocel (E10), Methocel (0.5 & E15) Hydroxypropyl methyl cellulose (HPMC) and Tween® 80) *via* single step ESp. Resulting fibrous films were evaluated for potential use in buccal delivery with increased amorphous drug content and a potential route to control drug volume and patch dimensions in a single step at the ambient temperature.

3.2 Aims and objectives

Co-polymer additives could influence the formulation and change the strength of intermolecular interaction among the formulation. Therefore, the aim of this chapter is to assess the effect of Methocel (0.5 and E15), Ethocel (E10), and HPMC with different concentrations (2 and 5% w/v) and Tween 80 with 5% which was added to the formulation with 5% w/v co-polymer formulations.

The obtained fibres were analysed by employing thermal analysis using differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA); spectroscopic analysis employing Fourier transform infrared (FT-IR) and Raman spectroscopy. The morphology and thickness of fibres were examined using Scanning Electron Microscopy (SEM) and atomic force microscopy (AFM). The sample preparation method was described in chapter 2.

3.3 Materials and methods

3.3.1 Materials

The information related to materials used in this chapter is in chapter 2 (method and materials)

Table 3. 1 Formulated fibres-sample compositions and their drug content.

Formulation	Polymer (5%w/v)	Drug (5% w/w)	Co-polymer (w/w)	Co-polymer (5% w/w)
1	Polyvinylpyrrolidone (PVP)	Indomethacin	-	-
2	Polyvinylpyrrolidone (PVP)	Indomethacin	Methocel (0.5) 2%	-
3	Polyvinylpyrrolidone (PVP)	Indomethacin	Methocel (0.5) 5%	-
4	Polyvinylpyrrolidone (PVP)	Indomethacin	Ethocel E10 2%	-
5	Polyvinylpyrrolidone (PVP)	Indomethacin	Ethocel E10 5%	-
6	Polyvinylpyrrolidone (PVP)	Indomethacin	Methocel E15 2%	-
7	Polyvinylpyrrolidone (PVP)	Indomethacin	Methocel E15 5%	-
8	Polyvinylpyrrolidone (PVP)	Indomethacin	hydroxypropyl methylcellulose (HPMC) 2%	-
9	Polyvinylpyrrolidone (PVP)	Indomethacin	hydroxypropyl methylcellulose (HPMC) 5%	-
10	Polyvinylpyrrolidone (PVP)	Indomethacin	Methocel (0.5) 5%	Tween 80
11	Polyvinylpyrrolidone (PVP)	Indomethacin	Ethocel E10 5%	Tween 80
12	Polyvinylpyrrolidone (PVP)	Indomethacin	Methocel E15 5%	Tween 80
13	Polyvinylpyrrolidone (PVP)	Indomethacin	hydroxypropyl methylcellulose (HPMC) 5%	Tween 80

3.3.2 Methods

Fibres preparation method is as same as the method mentioned in chapter 2 (methods and materials). All preparations contained PVP (5% w/v) and INDO (5% w/w of PVP). Other excipients were added in quantities shown in Table 3. 1 as a function of w/w % of PVP.

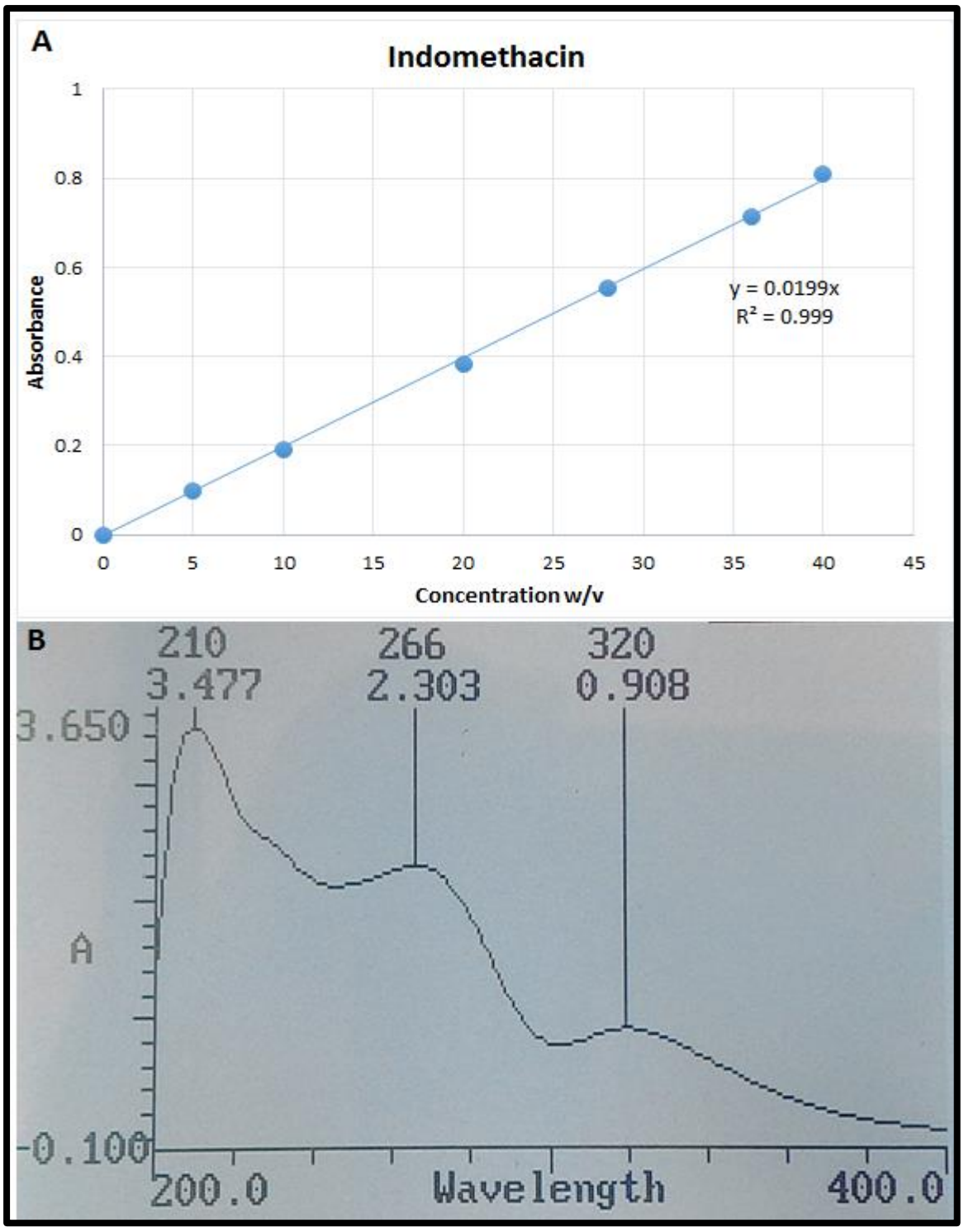


Figure 3. 2 UV-Vis calibration curve of Indomethacin in PBS at ambient temperature

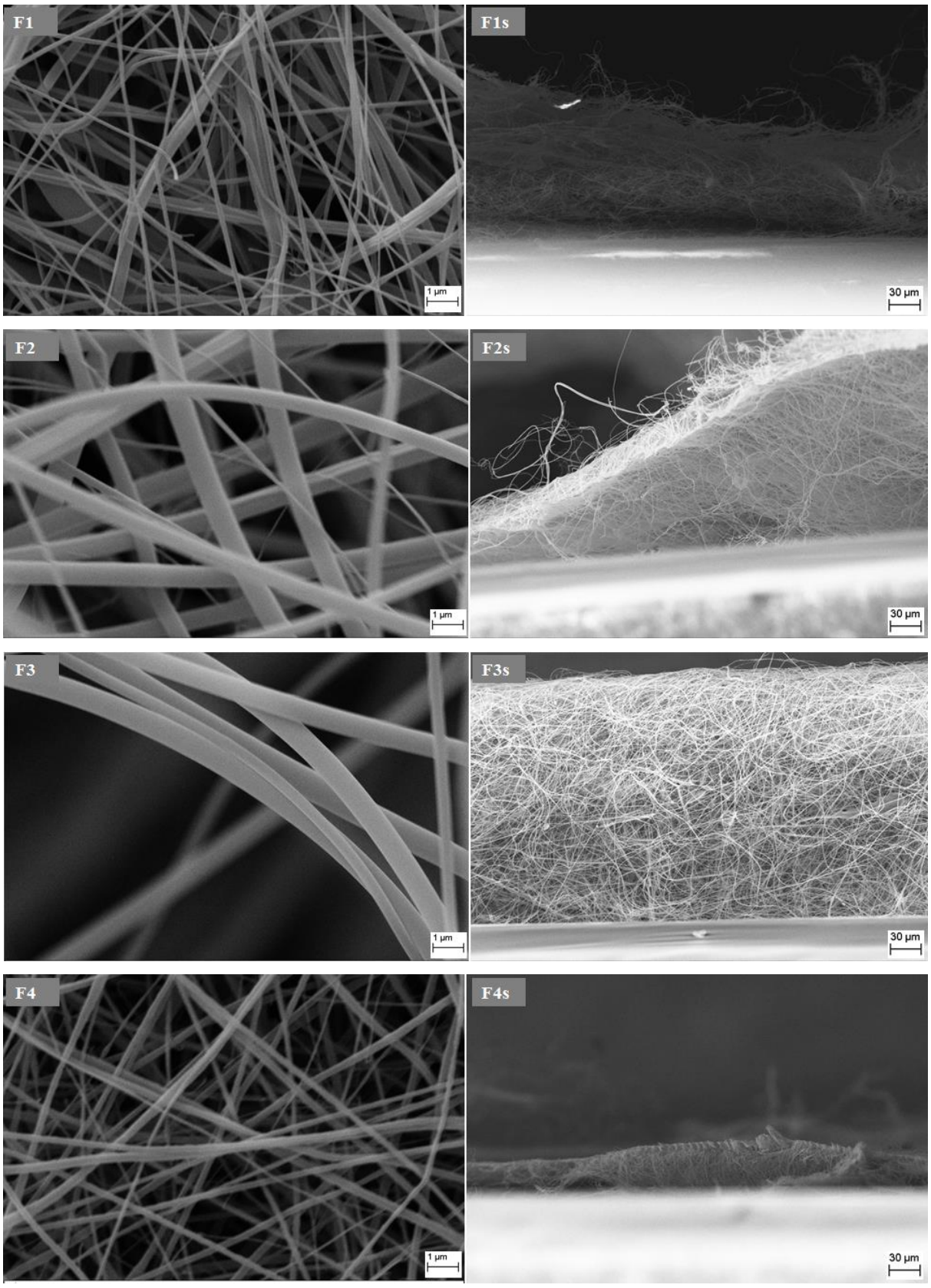
3.4 Results and discussions

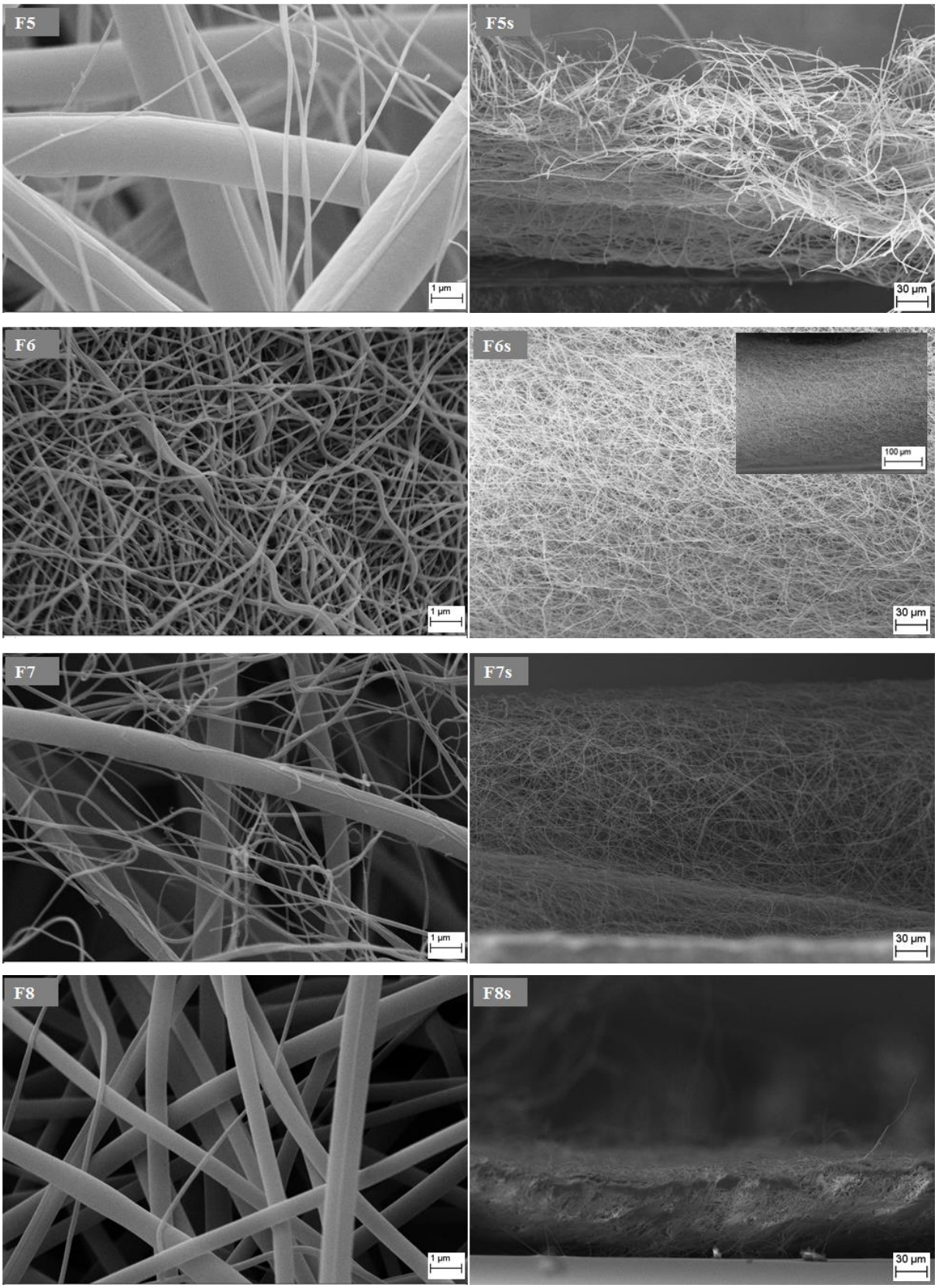
Figure 3. 3 shows the surface morphology of formulated electrospun films. Each film sample was imaged for plan and horizontal (width) analysis. All fibres exhibit smooth surface morphology with evident variation in fibre size distribution between samples. These arise due to formulation composition (e.g. co-polymers). Furthermore, the mean fibre diameter was found to increase with increasing co-polymer concentration in formulation from

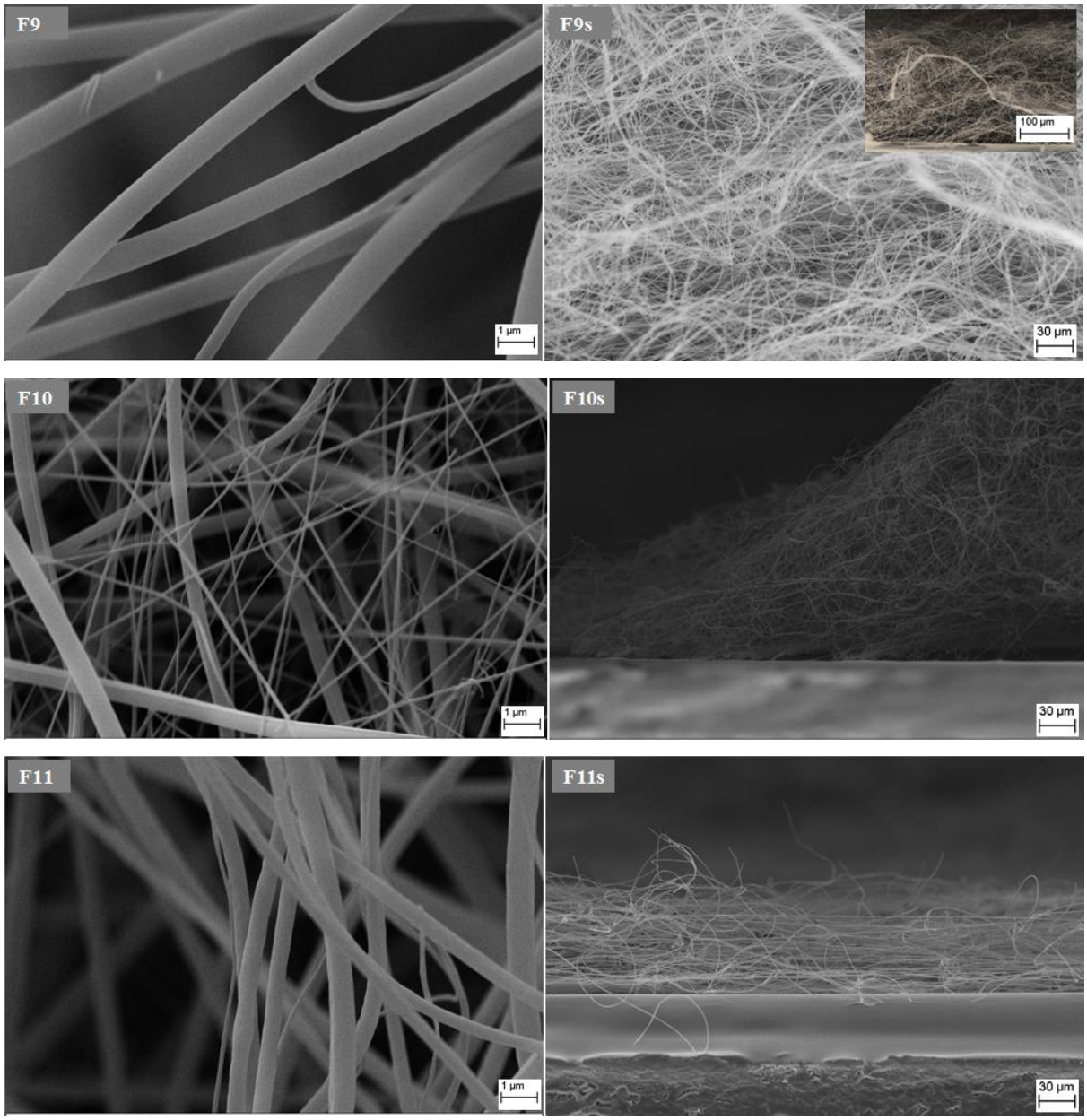
2% w/v to 5% w/v. Although the percentage of increasing in diameter is different for each co-polymer. Figure 3. 4 shows diameter size distribution histogram of 100 fibres for each formulated system. A clear trend is observed relating to pre-processed formulation media.

Fibres produced using Methocel (0.5) 2 w/v% (Figure 3. 4A) shows mostly in range of <900 nm which with increasing the percentage of co-polymer the diameter distribution has increased. On the other hand, F10 (5 w/v% + T80) shows the Fibres diameter distribution decreased and mostly <300 nm. Fibres produced using Ethocel (E10, 2 w/v%) (Figure 3. 4B) display a narrow diameter distribution (all fibres < 300 nm). Fibres produced using Methocel (E15, 2 w/v%) (Figure 3C) display a narrow diameter distribution same as formulation with Ethocel E10 (all fibres < 300 nm). A broader fibre diameter distribution is observed when using other **co-**polymers, especially HPMC 2 w/v% (Figure 3. 4D). A bell-shaped diameter distribution was observed for samples F8, F9 and F13.

Figure 3. 5 shows the side thickness (µm) of formulated electrospun films over predefined time (1h). Each film sample was imaged for side measurement analysis. F6 and F9 produced the thickest films. F1, F2, F4, F5, F8, F10 and F11 display a thin side diameter of less than 100 µm. This observation is associated with greater incorporation of drug (INDO) within fibres, which accordingly leads to enlarged fibre size (El-Naggar et al., 2016). Figure 3. 6 shows an AFM image of randomly selected fibres produced using formulation F5; PVP/INDO/Ethocel (E10). Deposited fibres display ribbon-like structures which arise due to two reasons. Firstly, the presence of residual solvent in filamentous structures leads to deformation upon contact with the collecting surface. Secondly, due to the hygroscopic nature of PVP, moisture absorption from the surrounding environment subsequently over time causes change to the fibre structure (Rasekh et al., 2014).


F1
1 µm
F1s
30 µm
F2
1 µm
F2s
30 µm
F3
1 µm
F3s
30 µm
F4
1 µm
F4s
30 µm


F5
1 µm
F5s
30 µm
F6
1 µm
F6s
100 µm
30 µm
F7
1 µm
F7s
30 µm
F8
1 µm
F8s
30 µm


F9
1 µm
F9s
100 µm
30 µm
F10
1 µm
F10s
30 µm
F11
1 µm
F11s
30 µm

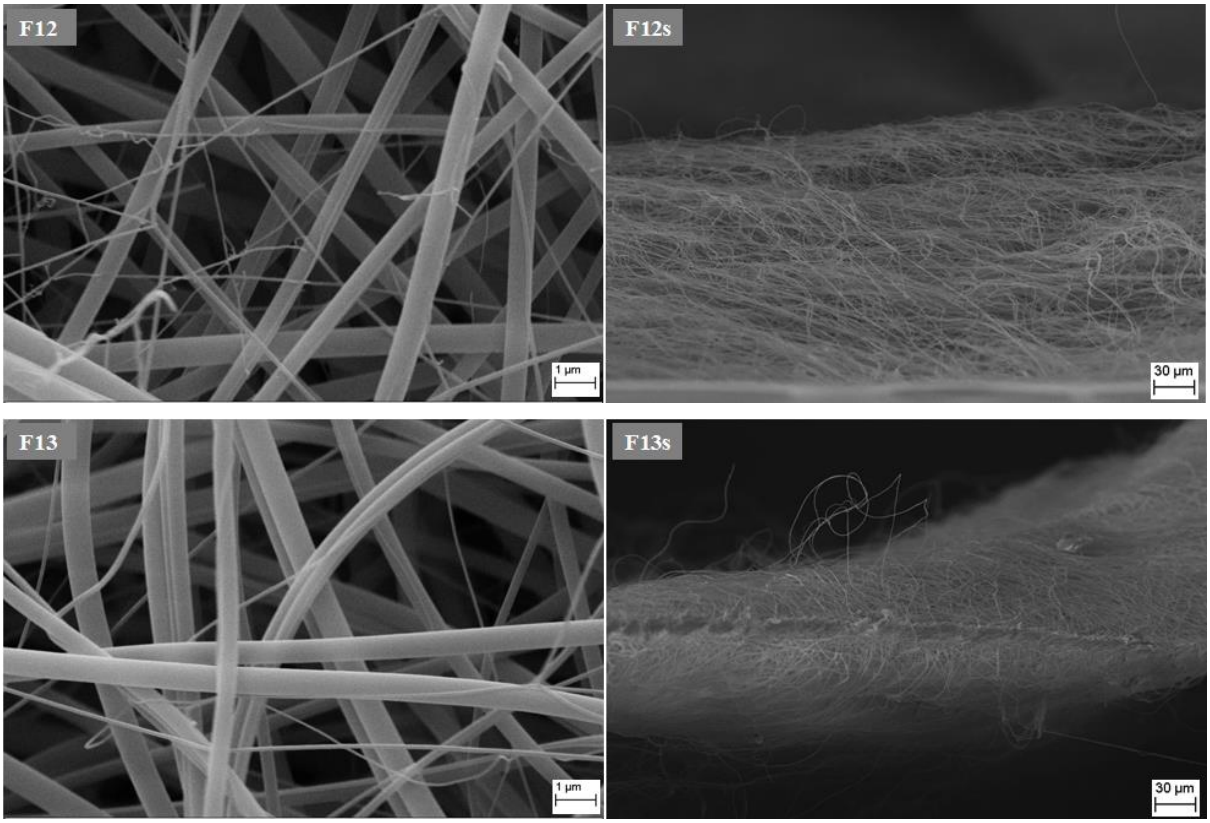
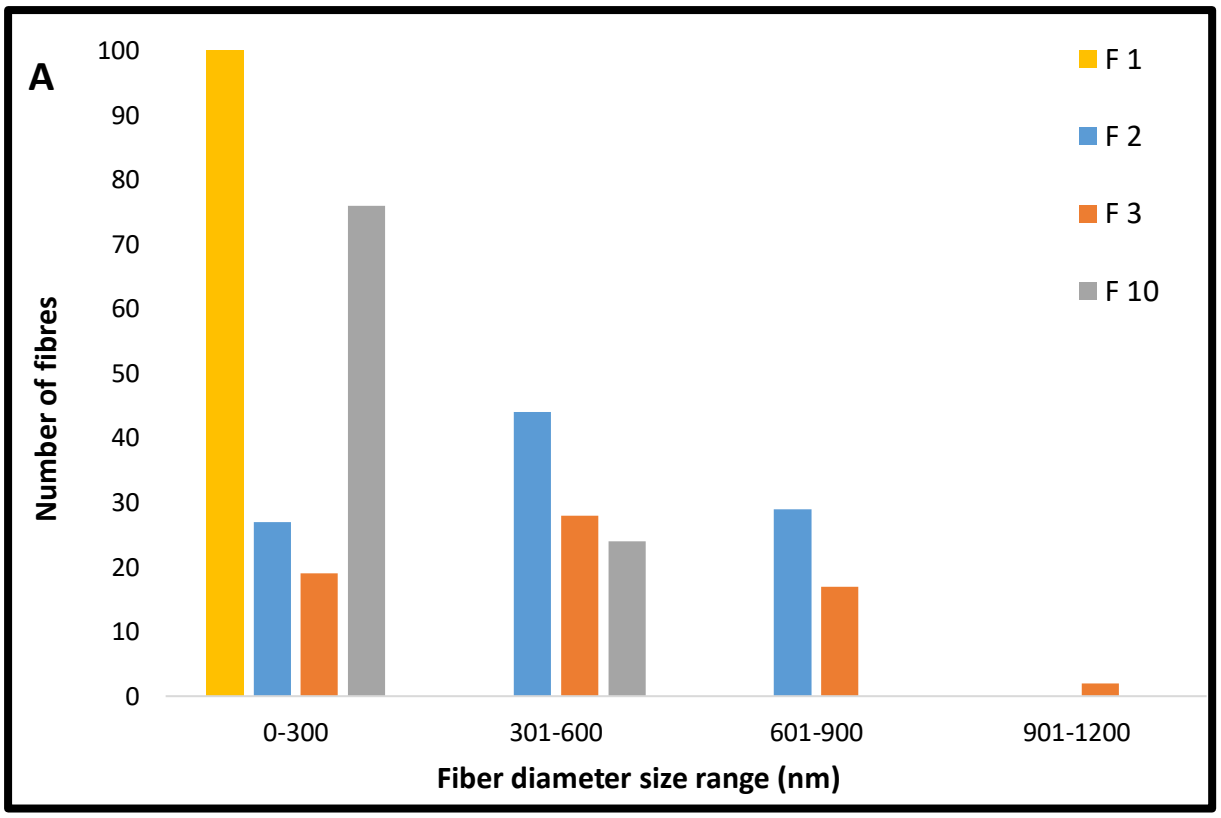
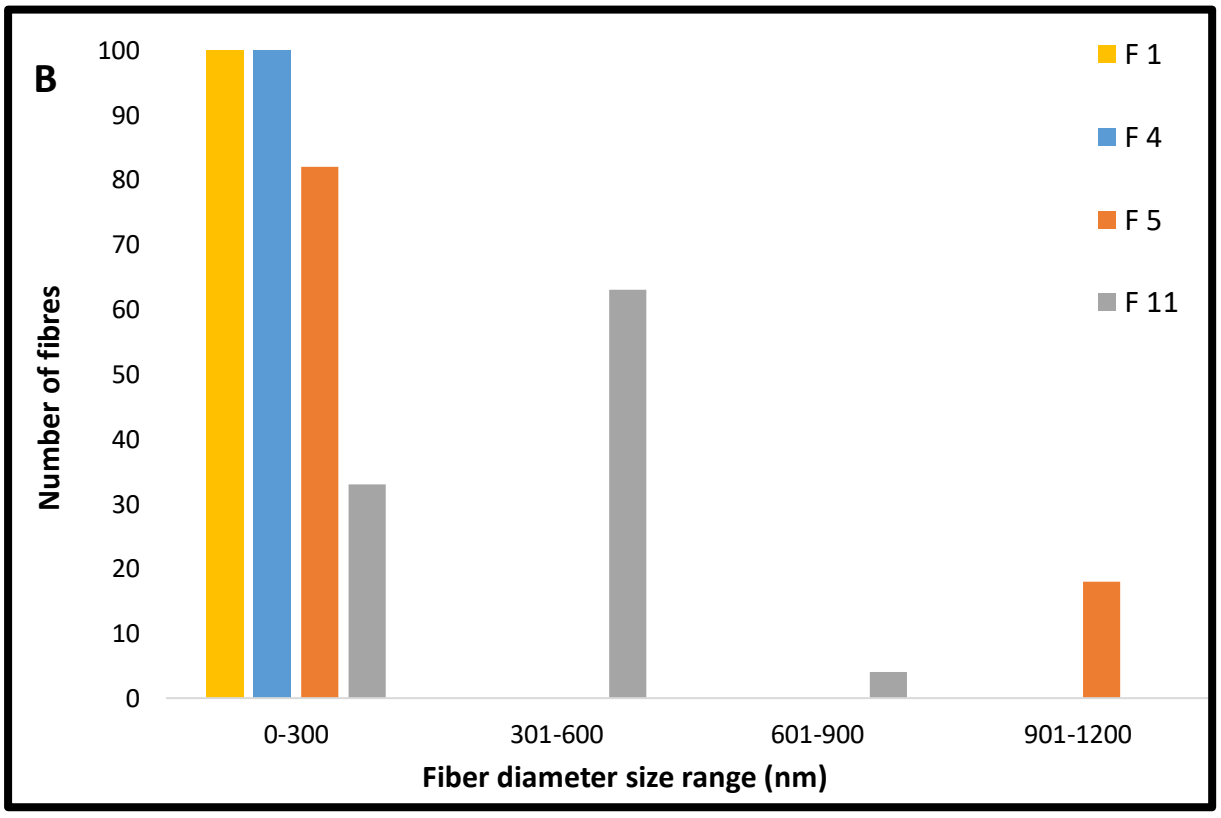
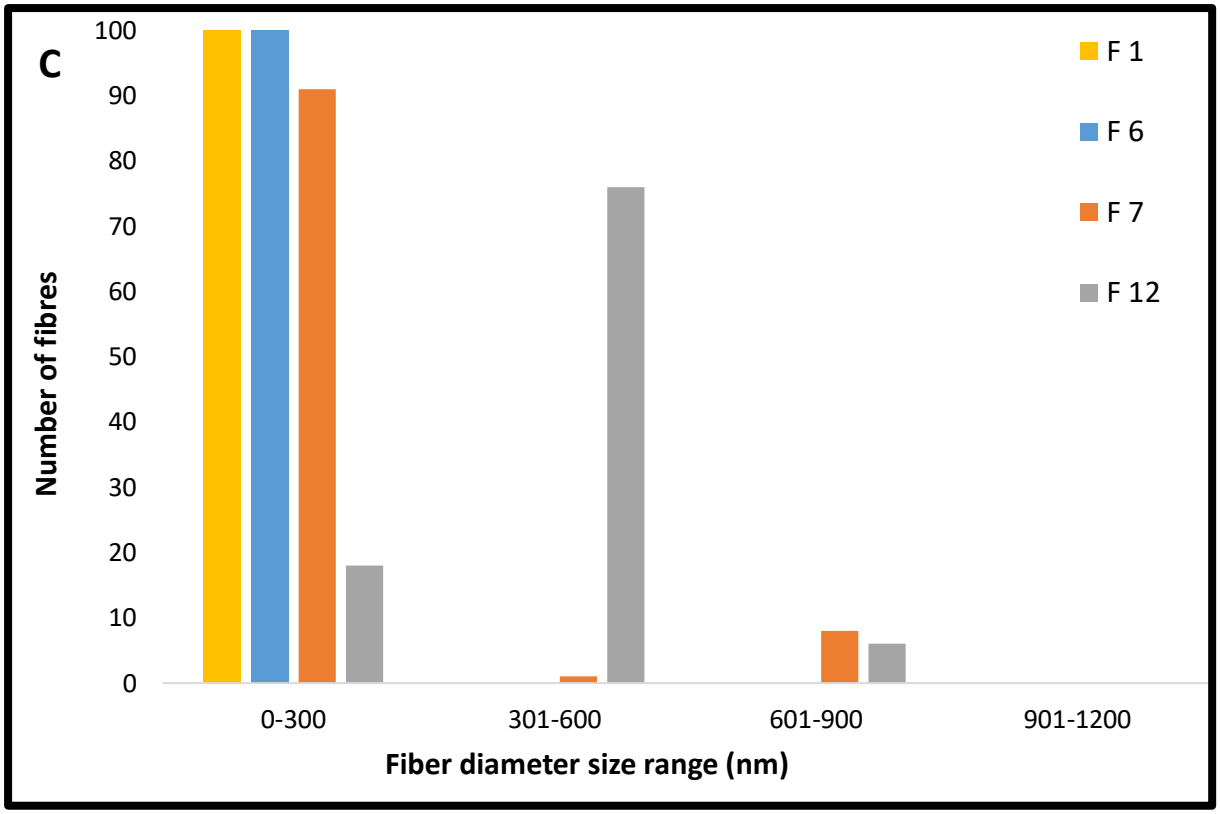


Figure 3. 3 SEM images of formulated fibres taken at X20K and side image X500 magnification.


A
Number of fibres
100
90
80
70
60
50
40
30
20
10
0
0-300
301-600
601-900
901-1200
Fiber diameter size range (nm)
F 1
F 2
F 3
F 10


B
Number of fibres
100
90
80
70
60
50
40
30
20
10
0
0-300
301-600
601-900
901-1200
Fiber diameter size range (nm)
F 1
F 4
F 5
F 11



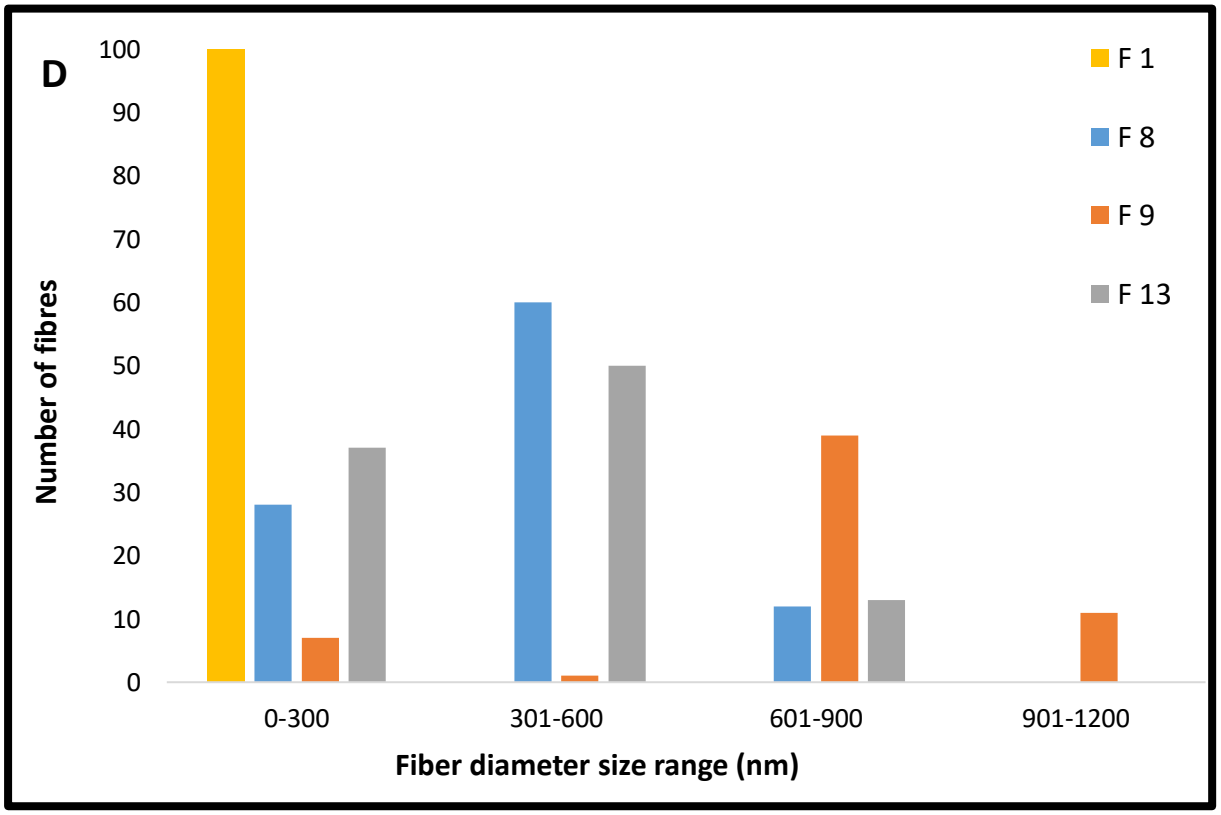


Figure 3. 4 Diameter size distribution histogram of 100 fibers of A (F1, F2, F3 and F10), B (F1, F4, F5 and F11), C (F1, F6, F7 and F12), and D (F1, F8, F9 and F13).

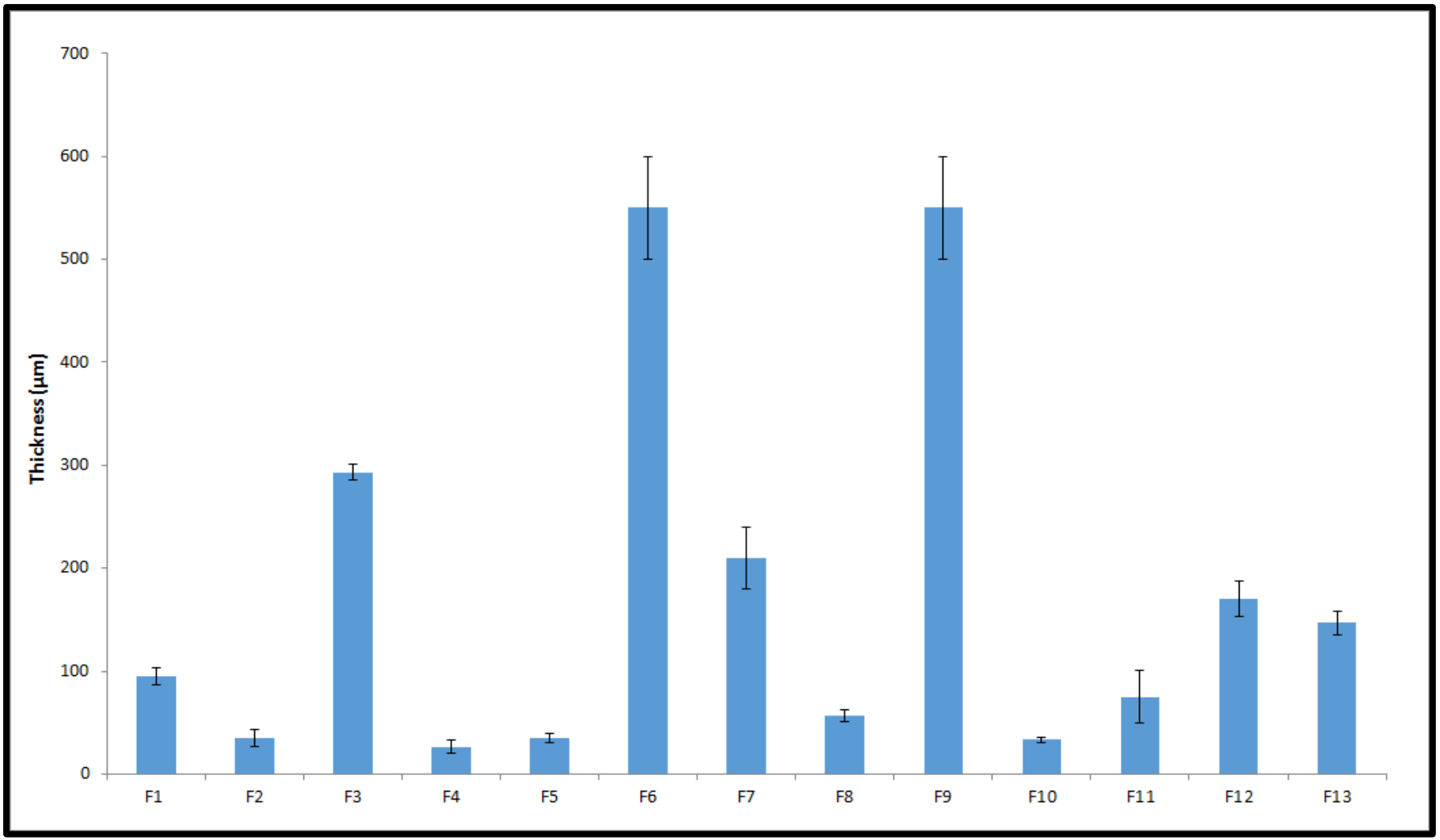


Figure 3. 5 Diameter size variation of Fibrous films side thickness (µm)

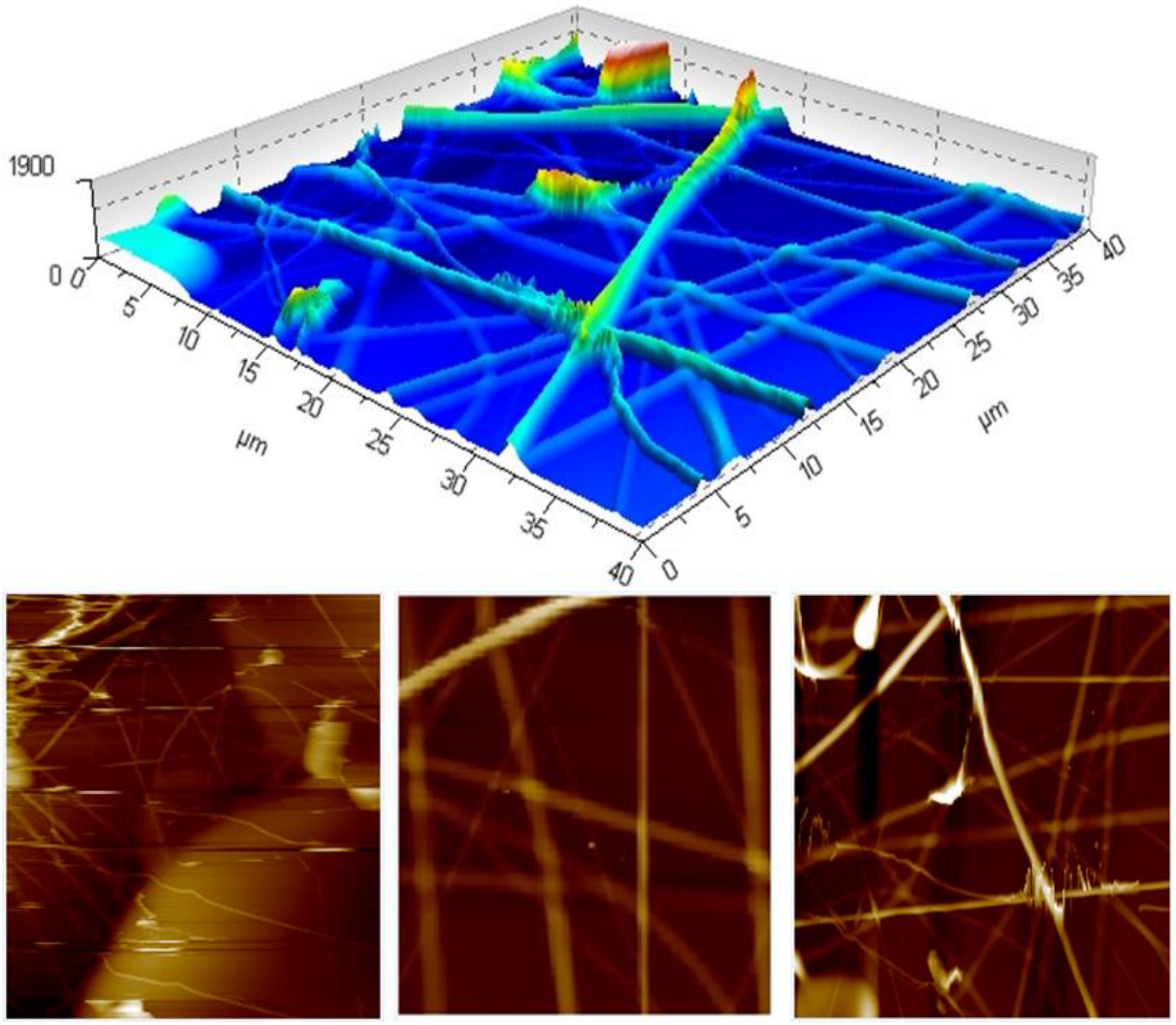


Figure 3. 6 AFM image of PVP/INDO/Ethocel (E10) (F5) fibrous films.

DCS thermograms are shown in Figure 3. 7A and Figure 3. 7B. INDO is a crystalline drug with a melting temperature ($\mathbf{T_m}$) of 163.5 °C which is shown at ~ 164 °C in Figure 3. 7B. The melting temperature of electrospun INDO/PVP films formulated and decreased significantly once compared to physical mixtures INDO/PVP, raw INDO and raw PVP. This difference corresponds to INDO being molecularly dispersed within the fibrous polymeric films increasing the amorphous state of the active. The melting point of electrospun films was higher for 2 w/w % polymer and reduced with increasing **co**-polymer content which might indicate increased amounts of polymer in the formulation providing a greater polymeric matrix volume for INDO dispersion and spacing. A clear difference is also noted between electrospun fibrous films (Figure 3. 7A) and physical mixtures and corresponding raw materials in the formulations (5B). Since the physical mixtures do not enable INDO dispersion as efficiently as electrospun

films, their melting temperatures are closer to the $\mathbf{T_m}$ values of neat drug. The combination of INDO, PVP, Ethocel (E10) 5% and Tween® 80 (F11) showed an exothermic peak around 145.32 °C which is credited to residual solvent entrapped within fibres.

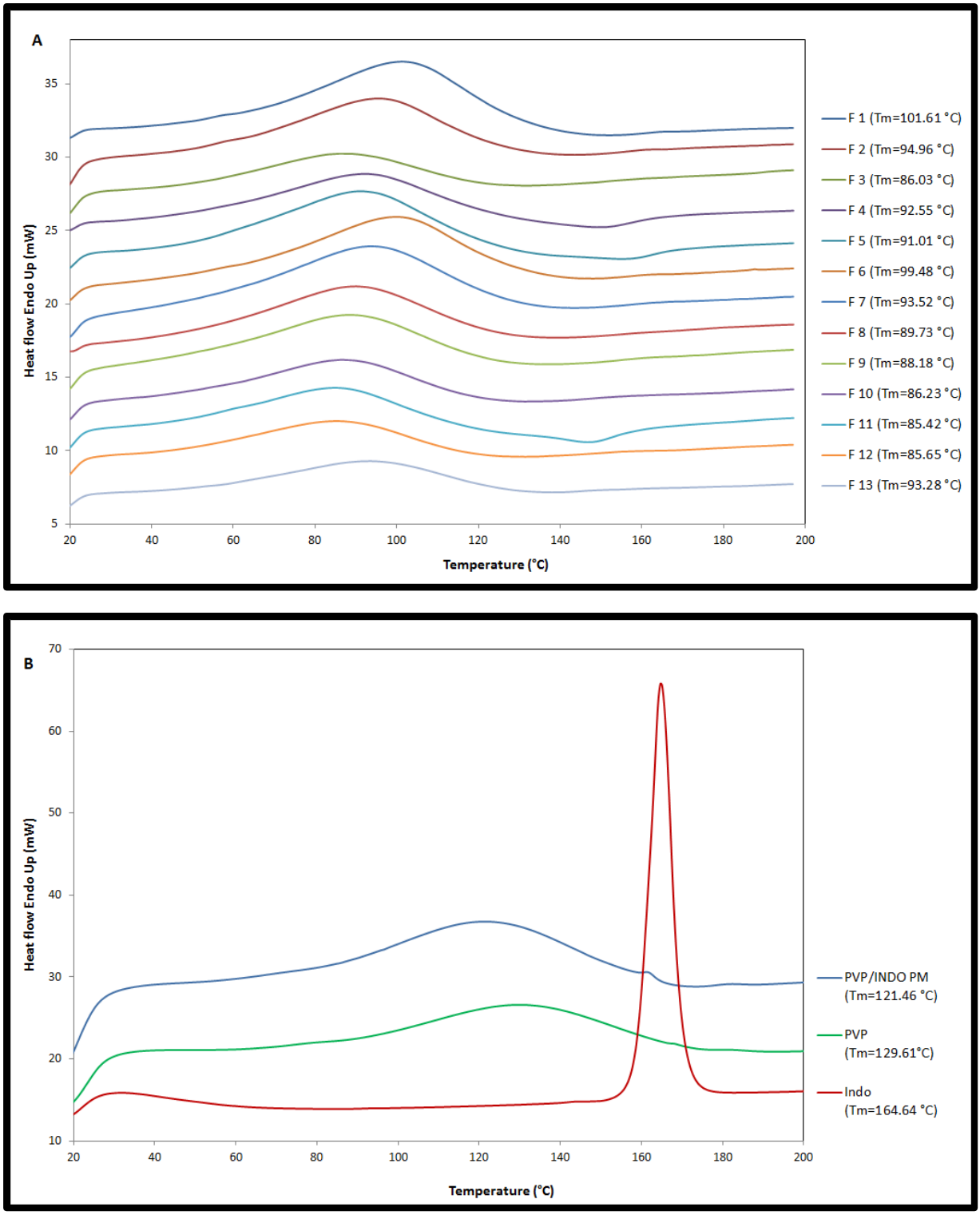
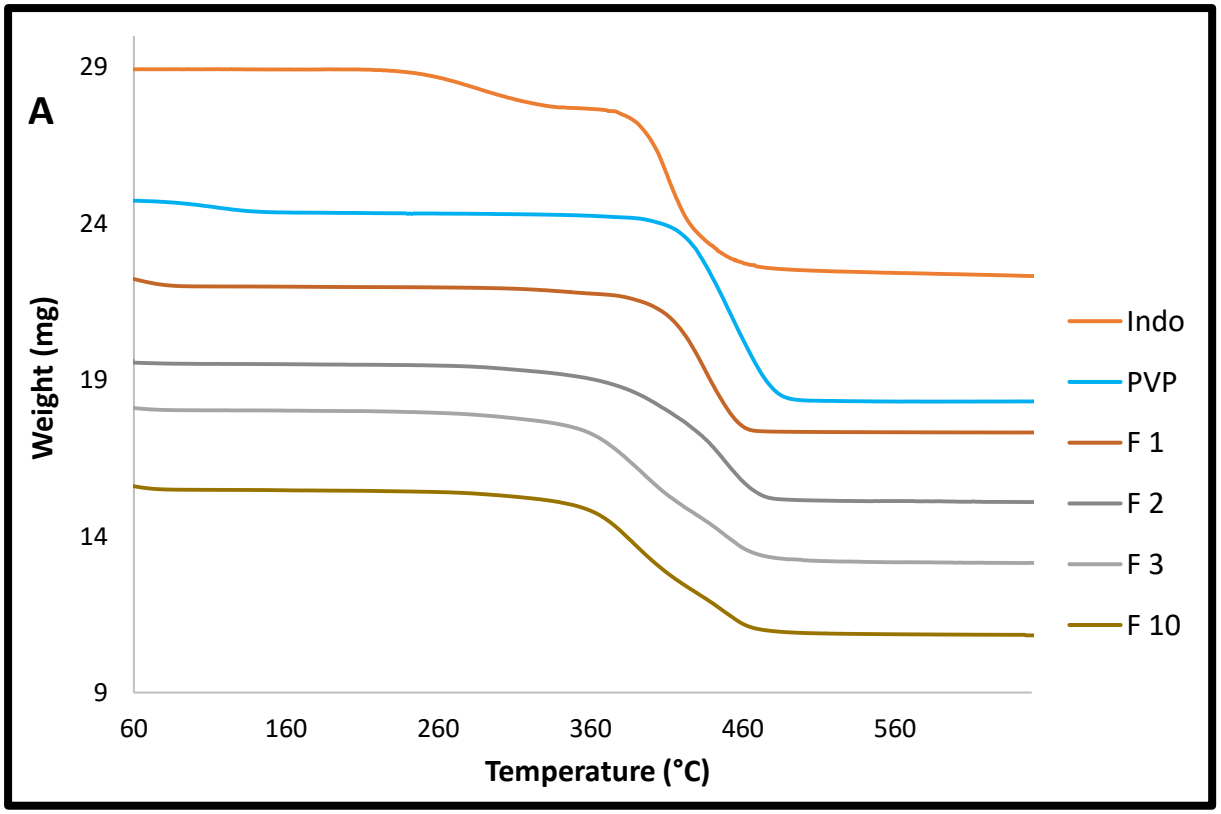
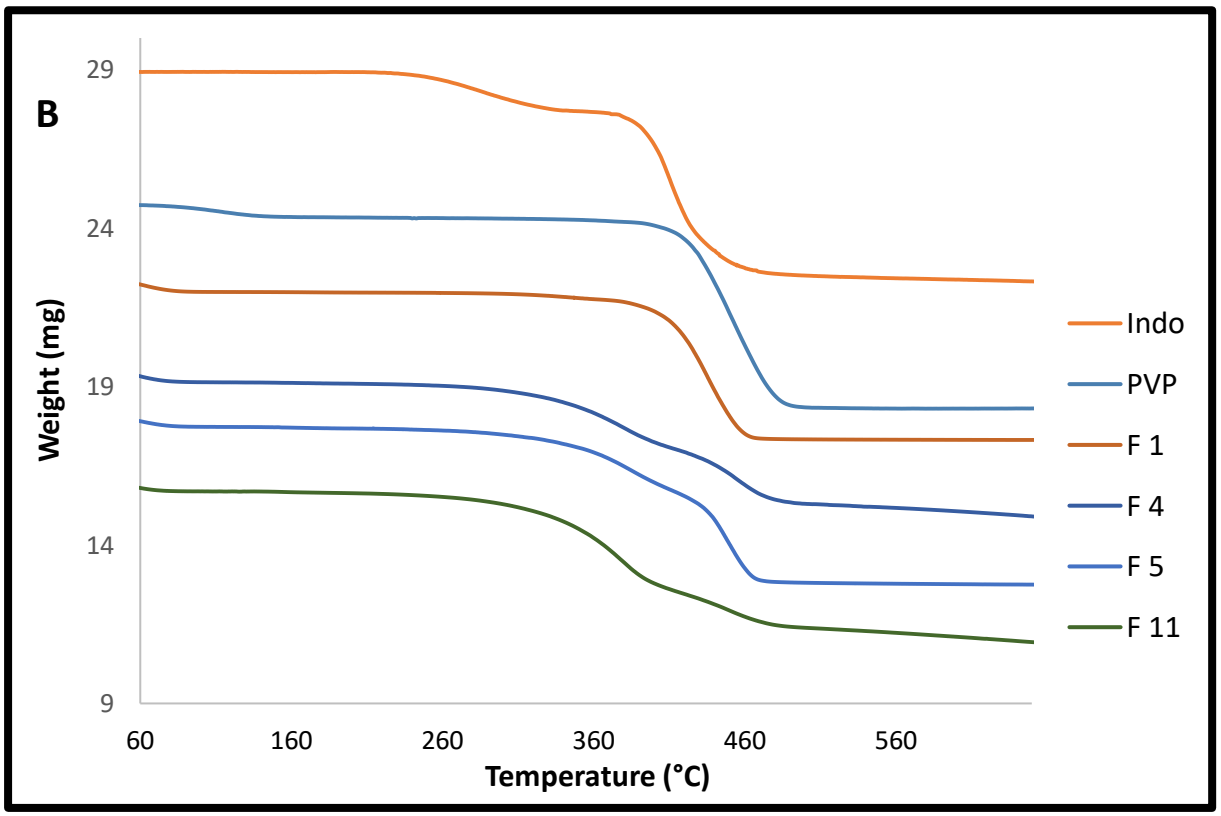


Figure 3. 7 DSC thermogram of A. formulated electrospun films B. raw PVP, INDO and physical mixture of PVP/INDO.

TGA was used to observe the thermal degradation behavior of fibrous films. Earlier studies have shown both neat electrospun samples and their drug loaded (INDO) counterparts to exhibit similar degradation steps (Taepaiboon, Rungsardthong and Supaphol, 2006). In this study, all formulations show two degradation steps; the first is weight loss observed at temperatures below 100 °C, which is attributed to the evaporation of the residual solvent or adsorbed water in fibrous films, The second major weight loss is observed from 350 to 500 °C, which is ascribed to the decomposition of PVP and other co-polymer excipients.


A
29
24
19
14
9
Weight (mg)
60
160
260
360
460
560
Temperature (°C)
Indo
PVP
F 1
F 2
F 3
F 10


B
29
24
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Weight (mg)
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Temperature (°C)
Indo
PVP
F 1
F 4
F 5
F 11

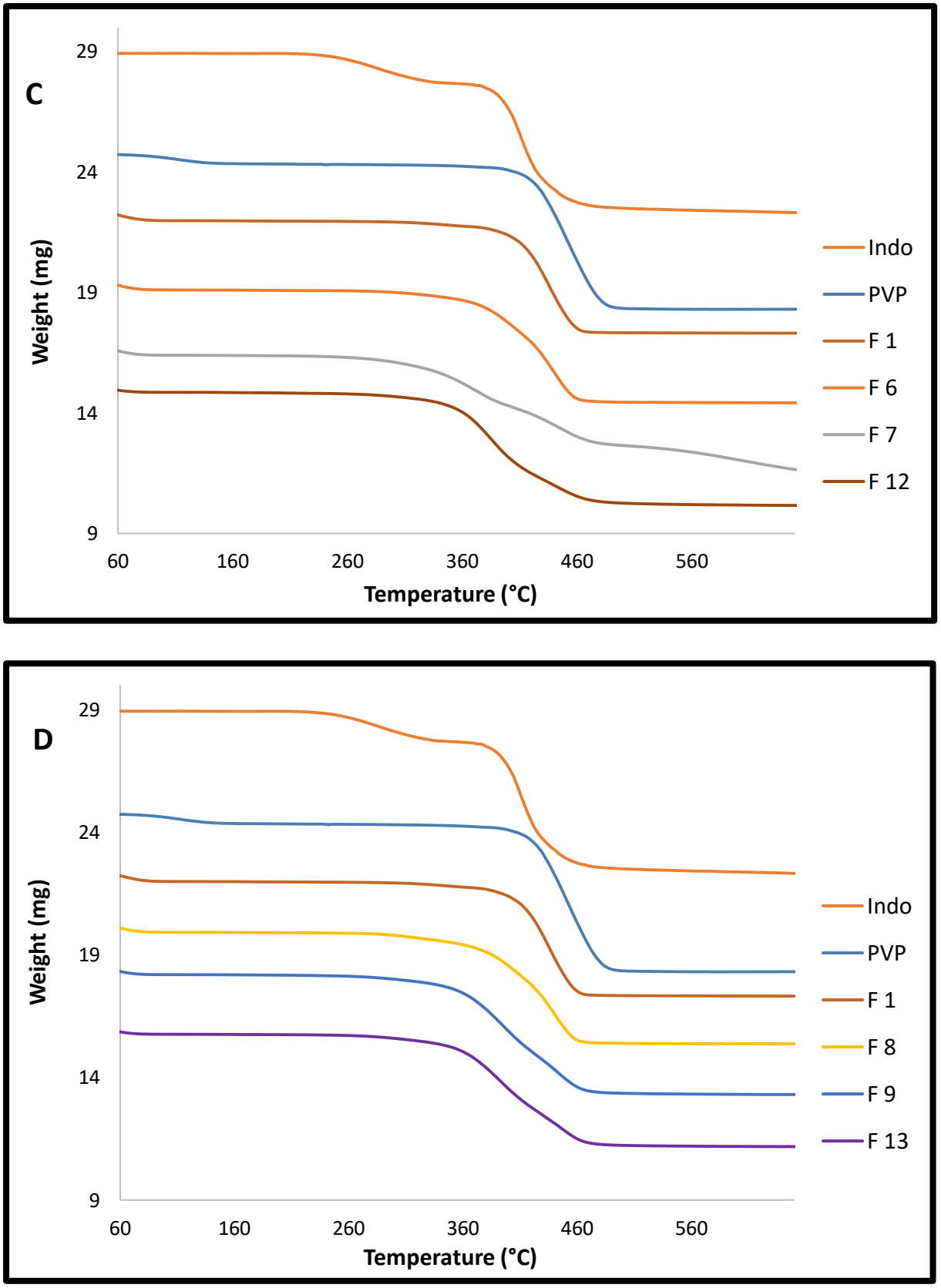
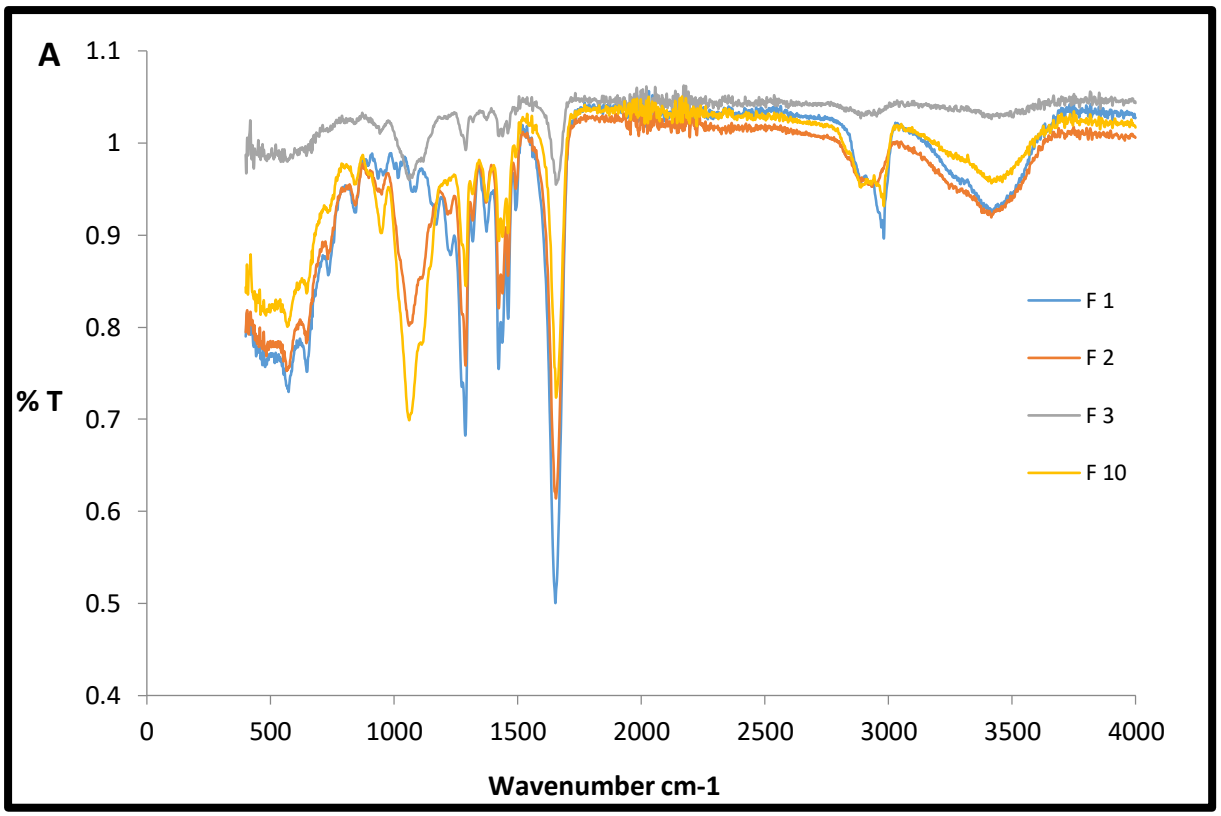
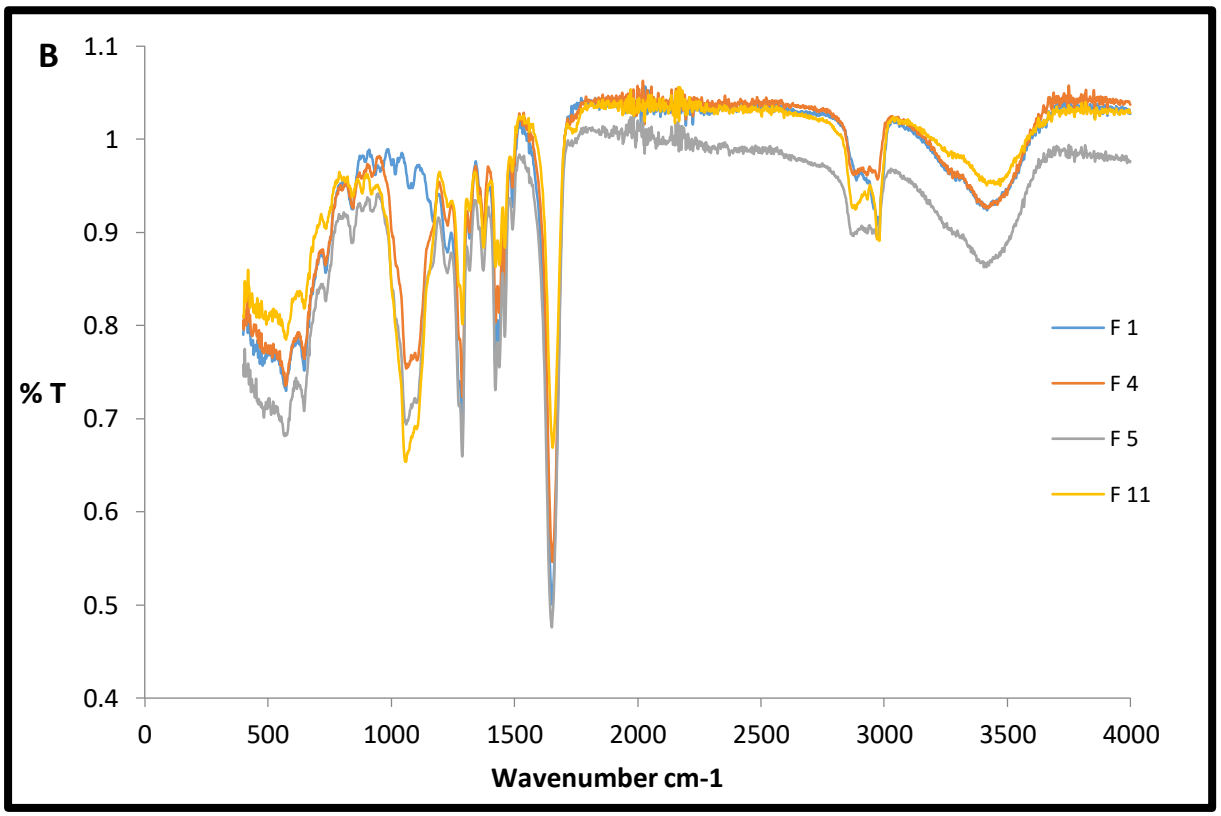


Figure 3. 8 TGA curves of formulated electrospun films.

TGA thermograms also confirm INDO loading into filamentous matrices. As has been reported in a previous study, a shift in the maximum temperature associated with weight loss can be used to demonstrate drug entrapment within a fibrous system (Taepaiboon, Rungsardthong and Supaphol, 2006). This has been demonstrated using several drugs including INDO, naproxen and diclofenac sodium; indicating thermal degradation can be expedited or delayed based on the selected active embedded within the fibrous film (Taepaiboon, Rungsardthong and Supaphol, 2006). As shown in Figure 3. 8, the maximum thermal degradation values of the various formulated drug loaded systems are different from neat INDO and PVP.


A
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
% T
0
500
1000
1500
2000
2500
3000
3500
4000
Wavenumber cm-1
F 1
F 2
F 3
F 10


B
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
% T
0
500
1000
1500
2000
2500
3000
3500
4000
Wavenumber cm-1
F 1
F 4
F 5
F 11

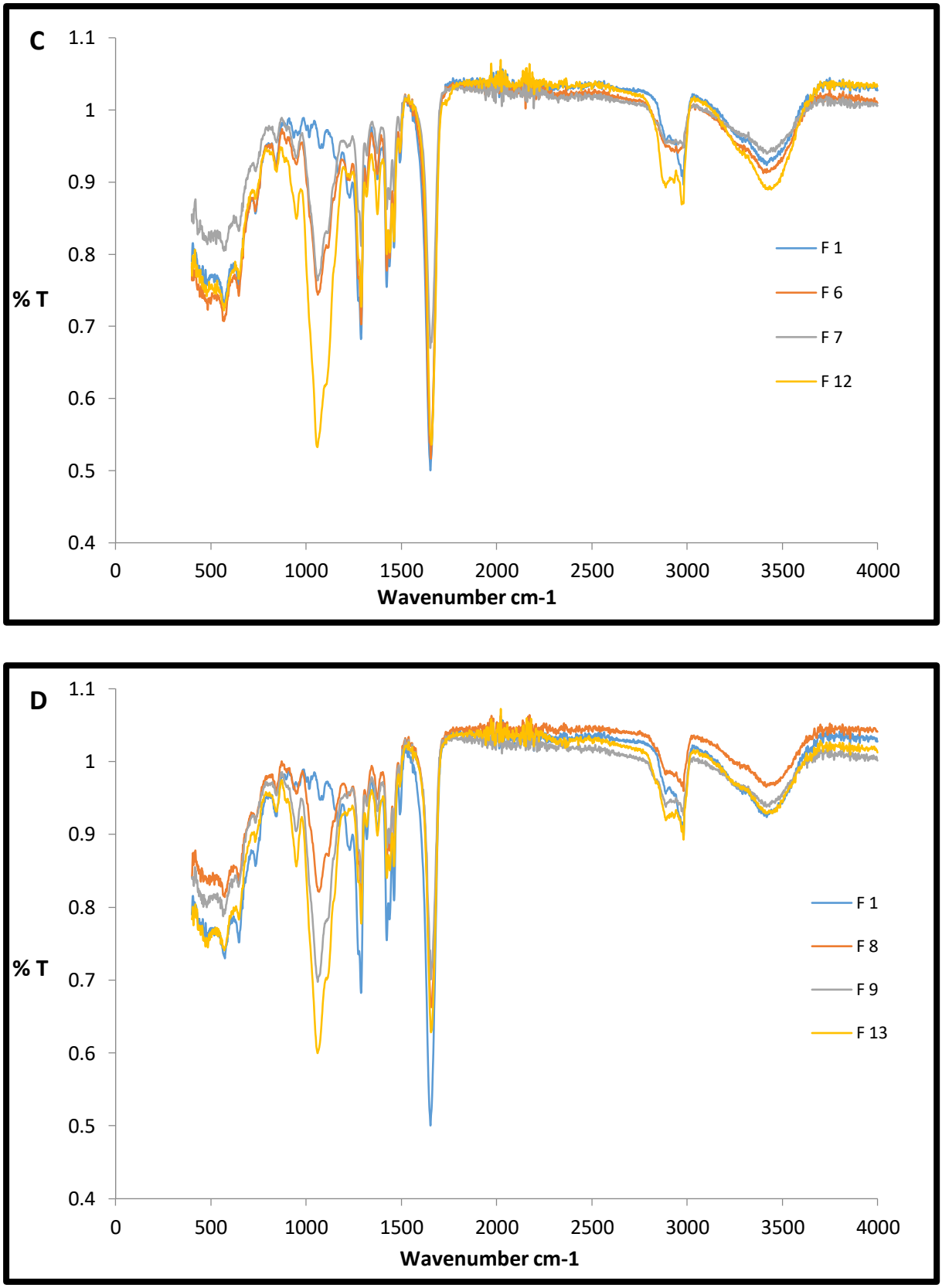
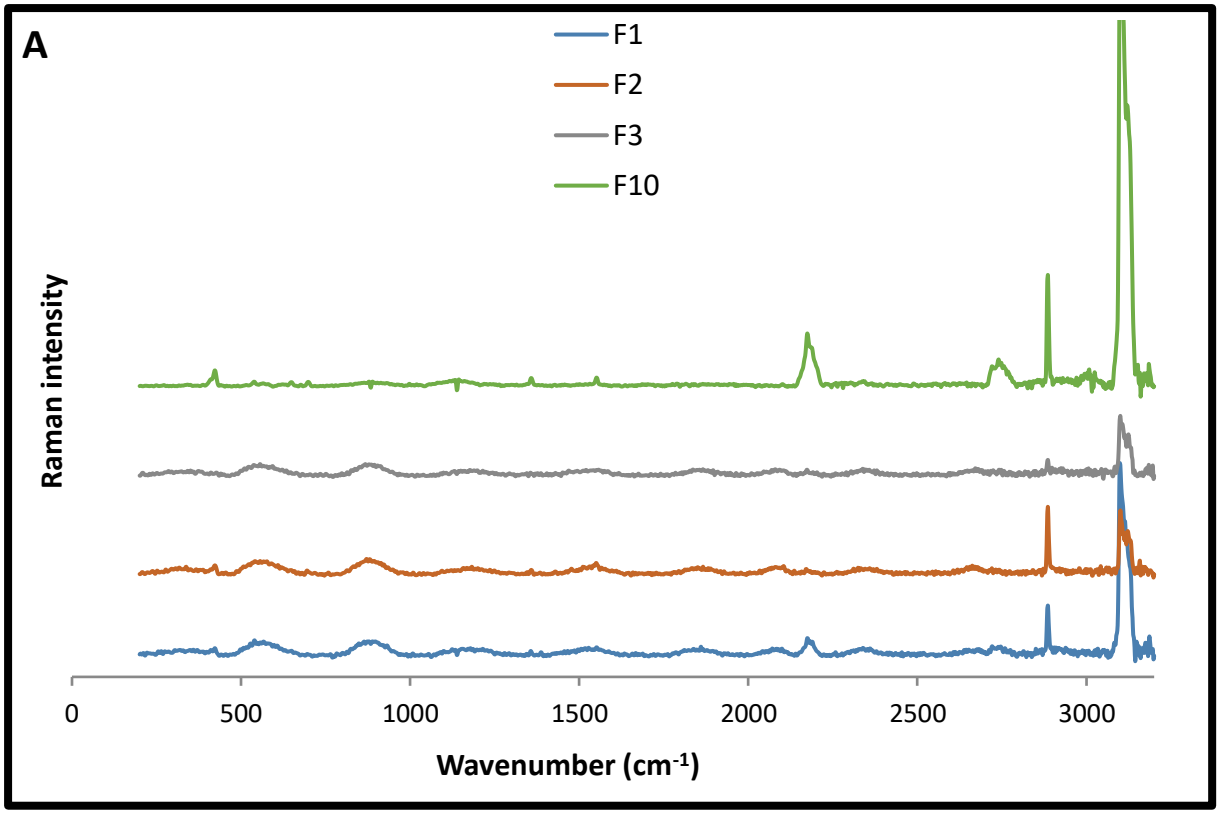
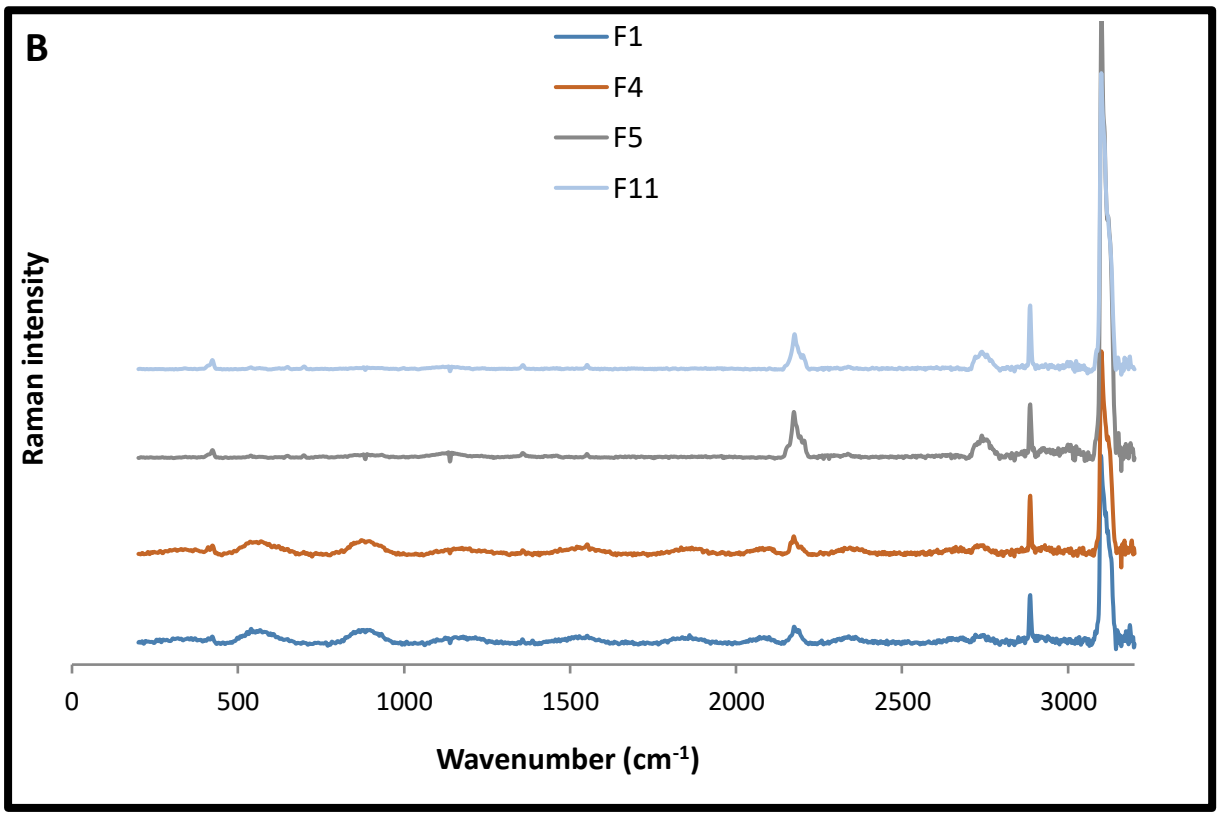


Figure 3. 9 FTIR spectra of formulated films.

Figure 3. 9A shows FTIR spectra for various electrospun samples with different co-polymer concentrations in the range 4000–400 cm^{-1}. The FTIR spectra of samples comprising INDO, PVP and other polymers show various characteristic peaks. These are attributed to vibration modes of the aromatic ring in INDO (particularly amide **ν**C═O at 1600 cm^{-1}), various peaks of PVP particularly the **ν**C═O at 1680 cm^{-1} which shifted to 1654 cm^{-1} as a result of hydrogen bond formation (Borodko et al., 2006). Other absorption peaks are recorded at 3490 (O—H), 2983 (C—H), 1920 (C═C) and 1425 cm^{-1} (C═C).

The changes observed in the INDO, PVP and co-polymer sample spectrum exhibit near identical peaks as INDO and PVP with a slight change to peak intensity at 1654 cm^{-1}. This arises due to Van der Waals force of attraction or hydrogen bonding between co-polymers. New absorption bands were detected in formulations containing co-polymers; apparent at 1087–1113 cm^{-1} and these are attributed to carboxylic acid carbonyl stretching in co-polymers.


A
F1
F2
F3
F10
Raman intensity
0
500
1000
1500
2000
2500
3000
Wavenumber (cm-1)


B
F1
F4
F5
F11
Raman intensity
0
500
1000
1500
2000
2500
3000
Wavenumber (cm-1)

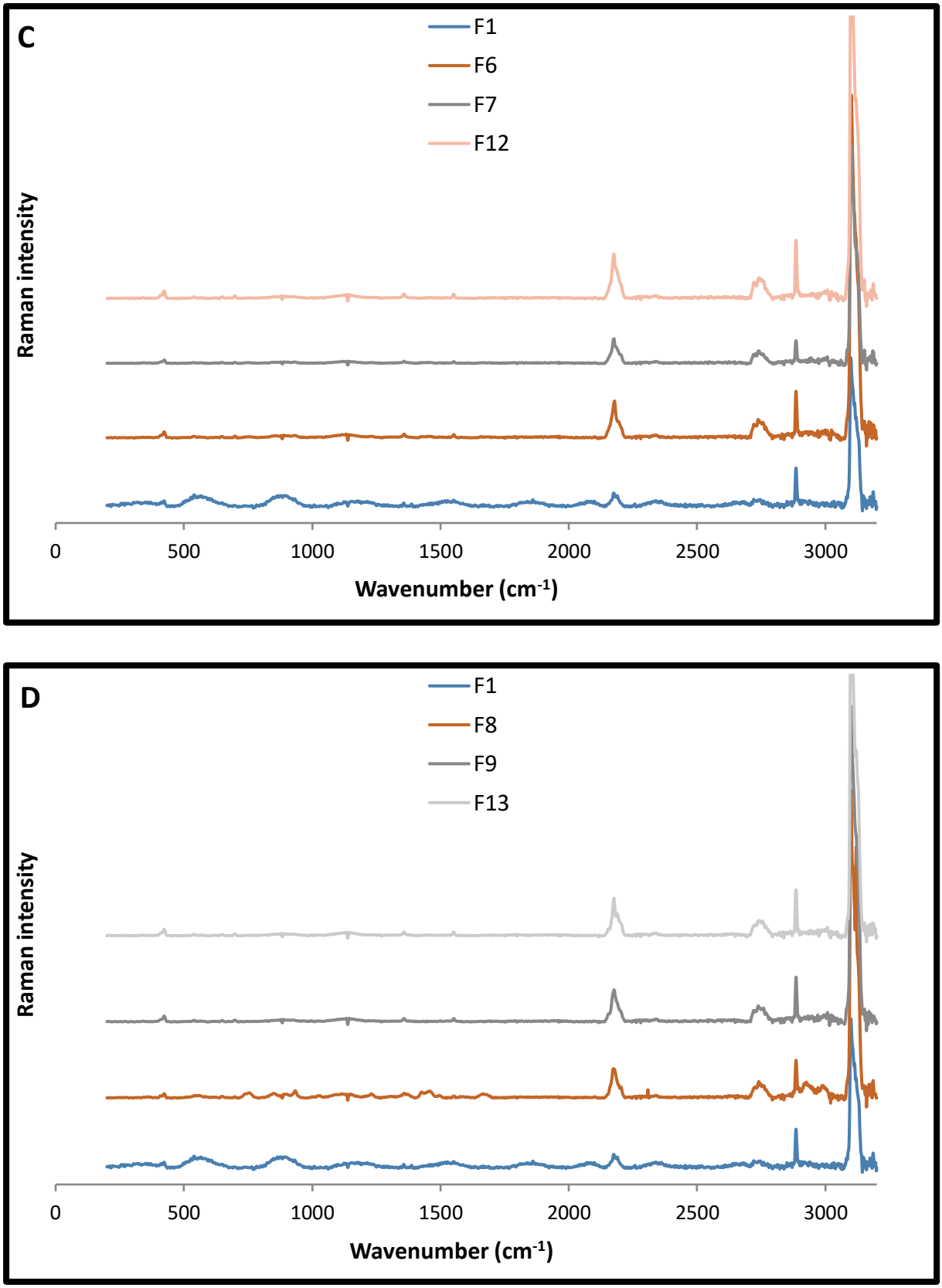


Figure 3. 10 Raman spectra of formulated films.

The FT-Raman spectra of samples comprising INDO, PVP and co-polymers are shown in Figure 3. 10. The FT-Raman spectra show a sharp peak at 3080 cm^{-1} which indicates O—H vibrational stretching of amorphous INDO carboxylic acid group. Other peaks were observed at 2884 cm^{-1} (**ν**(C—H)) and 2220 cm^{-1} (**ν**(C ≅ N)). The peak intensity at 3072 cm^{-1} increased for all formulations incorporating **co**-polymers. The increase in peak intensity is due to the increasing amorphous INDO content. Furthermore, a new band at 2760–2770 cm^{-1} (corresponding to co-polymers) was observed, suggesting that INDO, PVP and co-polymer system were all well integrated as a filamentous structure during ESp.

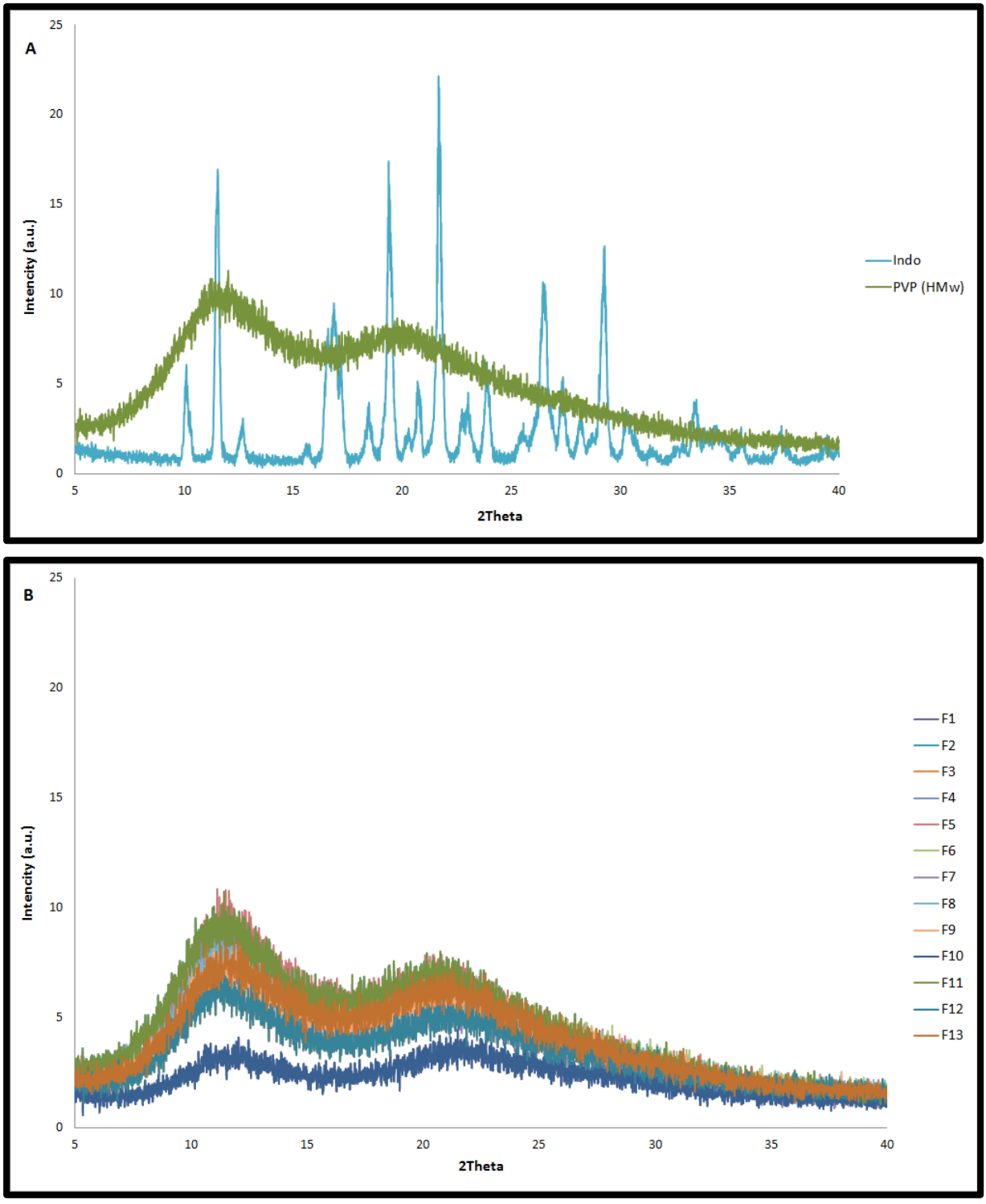
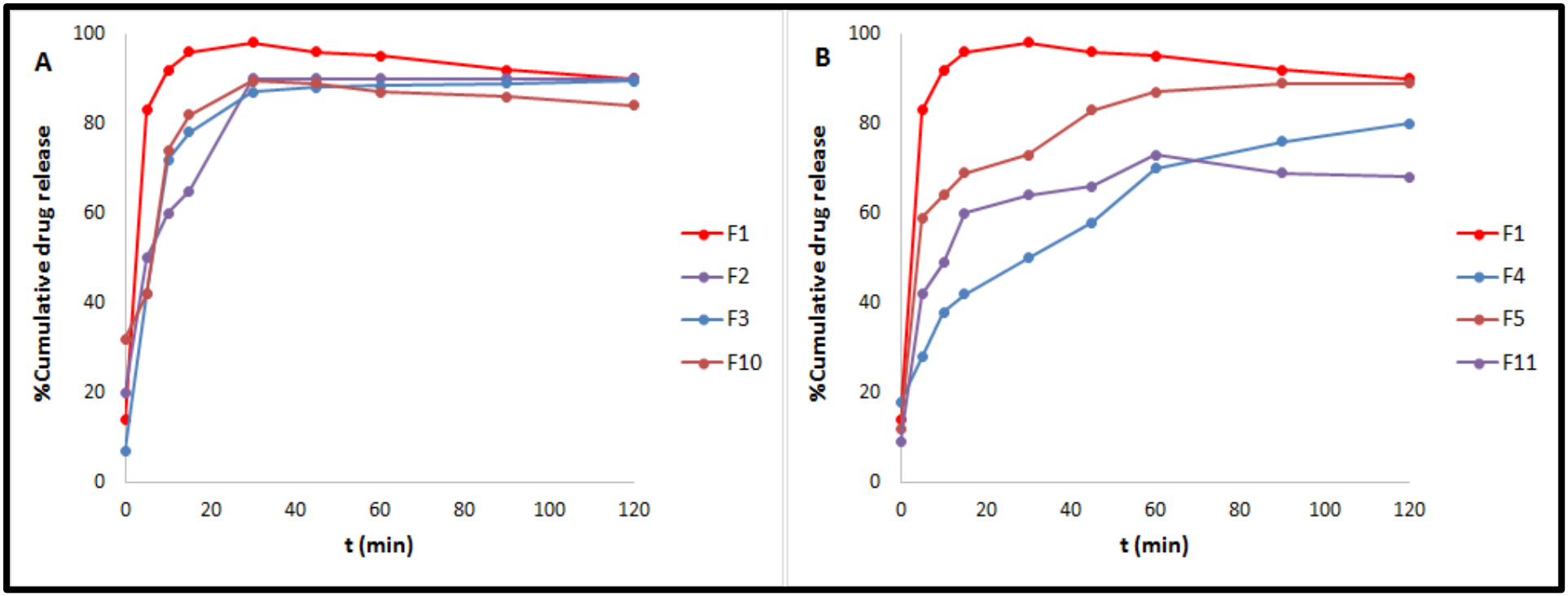


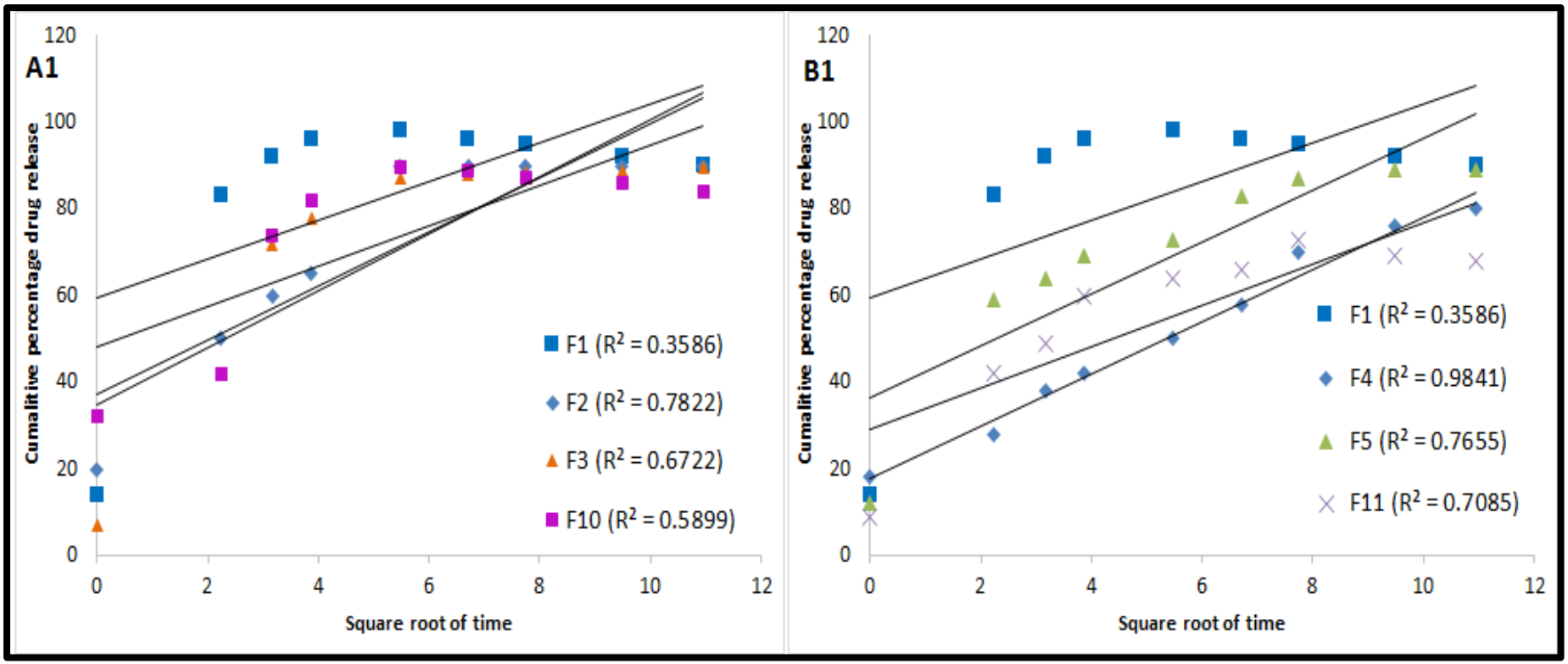
Figure 3. 11 X-ray diffractograms of A. INDO and PVP, B formulated electrospun films.

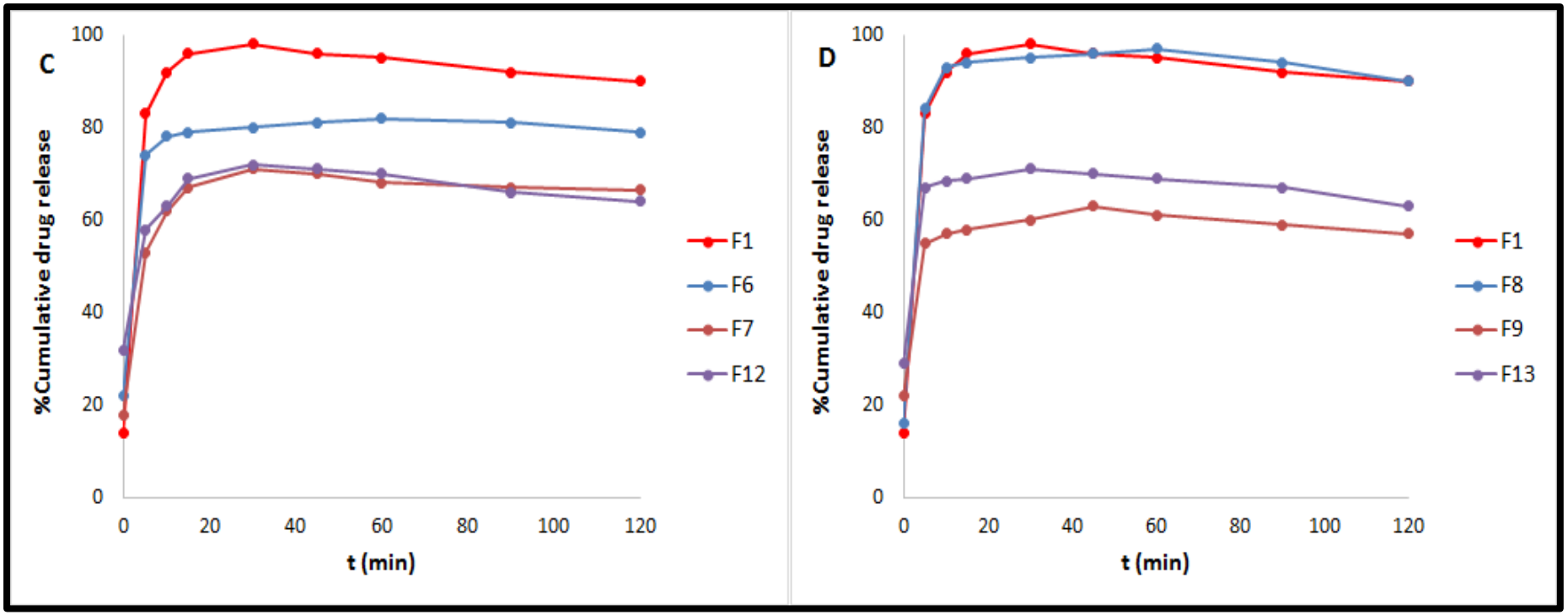
XRD patterns of formulated electrospun films are displayed in Figure 3. 11. The diffraction spectrum of pure INDO powder (Figure 3. 11A) demonstrates that the drug is highly crystalline and possesses multiple diffraction peaks at (~ 2 theta = 10–38°). All principle peaks of INDO

(11.7, 16.7, 19.5, 21.6 and 21.6) disappeared in the diffractogram of electrospun INDO with PVP and various other co-polymers. This indicates INDO is dispersed throughout the fibrous matrix and is in the amorphous state. The amorphous state provides several benefits and limitations for drug dosage forms. Integrating active in the amorphous state has shown to expedite drug release and bioavailability through enhanced solubility. However, this limits sustained release features and could also inadvertently result in crystallization and other stability issues.

The drug content in various film dosages ranged between 3 and 4.6% w/w which is less than the theoretical maximal value (5% w/w). This may be attributed to drug precipitation. Figure 3. 12A, B, C and D illustrate the cumulative release profiles for all formulated fibrous samples. A reduction in the release rate of INDO was observed by increasing the quantity of water insoluble polymer [Ethocel (E10)] from 2% (F4) to 5% (F5) in the formulated systems (Figure 3. 12B). The effect of ethyl-cellulose content on drug release from polymer beads containing INDO has been shown, where increased quantities of the polymer reduce the release rate of the active (Suryakusuma and Jun, 1984) (Suryakusuma and Jun 1984). This is explained due to the non-soluble nature of the material which limits water interaction with the polymeric matrix. The presence of Tween® 80 in samples increased INDO release rate when compared to its congener prepared with Ethocel (E10) 5% w/w (F5). Previously it has been shown that the solubility of INDO has been increased in the presence of the non-ionic surfactant Tween® 80 (ATTWOOD et al., 1989). A steep and fast release was recorded for all formulations within the first 5 minutes followed by a steady increase. Formulations with co-polymer Methocel 0.5 (Figure 3. 12A) showed fast release compared to other co-polymers and reached up to 90% of their release. On the other hand, formulation with co-polymer Ethocel E10 recorded slower release. Moreover, co-polymers Methocel E15 and HPMC indicated that with increasing the percentage from 2 w/v% to 5 w/v% release will be decreased Figure 3. 12C and D. The greatest release of INDO from fibres (~ 90%) was observed when 2% w/w HPMC was present in formulations, whereas a release of only ~ 58% was recorded for its congener with 5% w/w HPMC (Figure 3. 12D). A high HPMC polymer concentration results in the formation of a viscous gel-like layer which in turn impedes the diffusion of the drug towards the external layer and into the test medium.


A
%Cumulative drug release
t (min)
F1
F2
F3
F10
B
%Cumulative drug release
t (min)
F1
F4
F5
F11


A1
Cumalitive percentage drug release
Square root of time
F1 (R^2 = 0.3586)
F2 (R^2 = 0.7822)
F3 (R^2 = 0.6722)
F10 (R^2 = 0.5899)
B1
Cumalitive percentage drug release
Square root of time
F1 (R^2 = 0.3586)
F4 (R^2 = 0.9841)
F5 (R^2 = 0.7655)
F11 (R^2 = 0.7085)


C
%Cumulative drug release
t (min)
F1
F6
F7
F12
D
%Cumulative drug release
t (min)
F1
F8
F9
F13

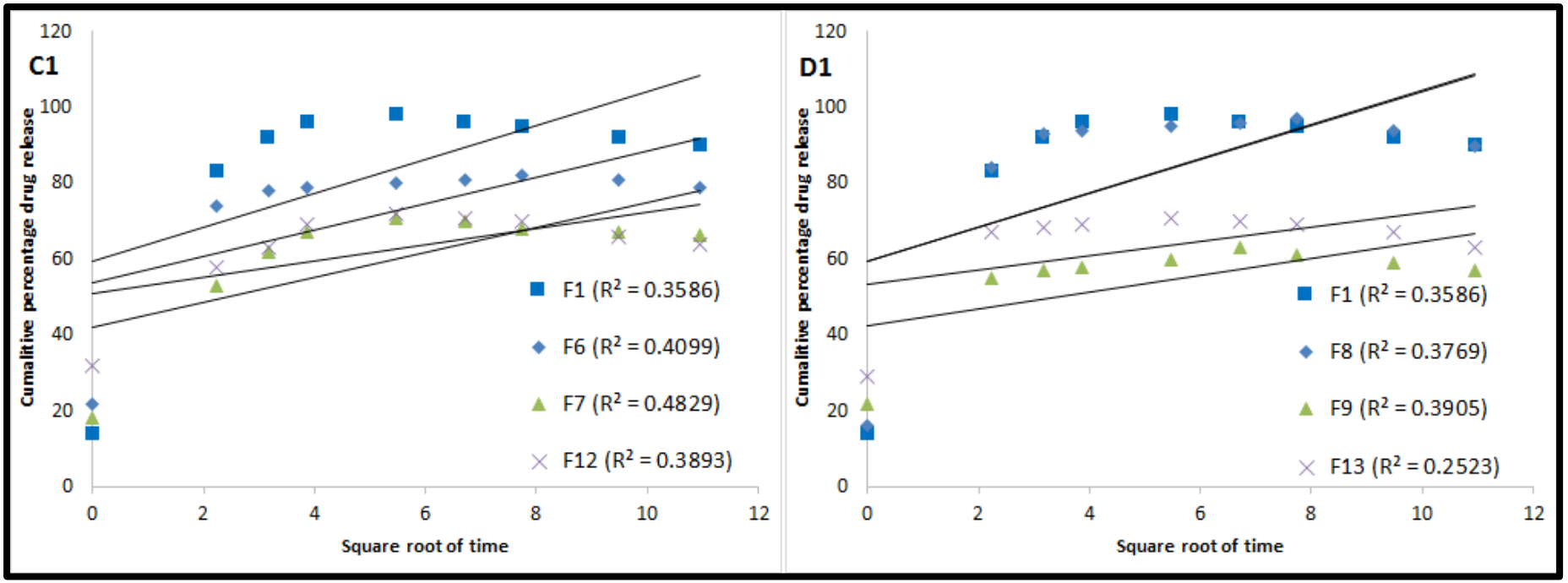


Figure 3. 12 Release profiles of A. F1, F2, F3, F10, B. F1, F4, F5, F11, C. F1, F6, F7, F12, and D. F1, F8, F9, F13 formulations in buffer pH 6.8. A1, B1, C1 and D1 show plots of cumulative release against root time for corresponding formulation.

The presence of Tween® 80 in fibres prepared with high concentration of HPMC (5% w/w) increased the dissolution rate of INDO (~ 62%) (Figure 3. 12D). The release of INDO from fibres containing HPMC exhibited a fast release in the first 5 min followed by a plateauing effect until the end of the study. The differences observed in INDO release behavior from fibres containing Methocel (0.5), Ethocel (E10), Methocel (E15) and HPMC is attributed to higher viscosity of Ethocel (E10) compared to that of Methocel (0.5), Methocel (E15), and HPMC resulting in prolonged release of the active over time. Furthermore, the release rate of INDO has been modulated previously using ethyl cellulose and HPMC in the formulation (Ohara et al., 2005).

The kinetic release model of PVP/INDO/co-polymer nanofibers is shown in Figure 3. 12A1, B1, C1 andD1, in which the cumulative quantity of drug released per square centimeter of fibrous films is plotted against time and fitted to the Higuchi model. It was observed that the release profile of INDO from fibrous samples of F4 (PVP/INDO/Ethocel (E10) 2%) (R^2 = 0.9841) suggest that the release of the drug in this formulation is governed by diffusive mechanisms. This implies INDO release is based on Fickian diffusion, based on drug permeation through the fibrous polymeric network (Neo et al., 2013).

3.5 Conclusion

In the current study an array of formulated films containing INDO were fabricated using electrospinning (Esp) technology. The drug associated with the fibres was in an amorphous state (molecularly dispersed) as evidenced from the X-ray studies whereas the release rate was mainly controlled by polymer composition in a sustained manner. The *in vitro* release of INDO was regulated by the quantity and the hydrophilicity/hydrophobicity of Methocel (0.5), Methocel (E15), HPMC and ethyl-cellulose in the formulation. The presence of the non-ionic surfactant Tween® 80 appears to have a synergistic effect with the polymers depending on their amount present in the formulations. The strategy proposed in this study provides a method to design novel INDO-polymer hybrid electrospun mats utilized for buccal delivery.

3.6 References

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FONSECA-SANTOS, B. and CHORILLI, M. (2018b) *An overview of polymeric dosage forms in buccal drug delivery: State of art, design of formulations and their in vivo performance evaluation* Available from: http://www.sciencedirect.com/science/article/pii/S0928493117331880.

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Chapter 4 Comparing fast and slow release

4.1 Introduction

Polymer selection for the design and development of drug dosage forms is as important as the nominated active pharmaceutical ingredient (API) (Ting et al., 2016). Formulation and selective use of excipients enable specific chemical and physical properties including manufacturing method, dosage stability, packaging, handling and bio-interface interactions. A vast variety of polymers, from both synthetic and naturally occurring classes, are used for commercial drug dosage manufacturing and optimization. Selection is often derived on the basis of material suitability when considering engineering steps during product development (e.g. including particle or fibre engineering (Hussain et al., 2006; Liechty et al., 2010).

The buccal route for API administration has gained popularity over the last decade due to advances in drug discovery (coupled with potential opportunistic therapies) (Repka, Chen and Chan, 2011; Mura et al., 2016), on-demand dosage form development and also to overcome disadvantages associated with conventional oral dosage forms (e.g. difficulty swallowing or tolerating the enteric route) (Lopez et al., 2015; Nazari et al., 2017). Several methods to develop or formulate buccal drug delivery systems have been explored through innovative developments in films, patches, tablets and even capsules (Abruzzo et al., 2012). Rapid drug absorption (from various dosage form types e.g. pill or film) has been shown in the buccal cavity, in addition to greater patient compliance compared with other oral transmucosal administration routes (Reddy et al., 2011). Furthermore, greater volume of vascularized tissue and blood vessels at this location indicate potential entry into systematic circulation (Pather, Rathbone and Senel, 2008), possibly through the jugular vein (Lopez et al., 2015; Reddy et al., 2011; Marxen et al., 2016). Avoiding drug hydrolysis (often encountered in the gastrointestinal tract), enzymatic and acidic action, as well as bypassing first-pass metabolism are all drivers for the development of buccal drug delivery systems (Reddy et al., 2011). In terms of interactions, a characteristic feature of the buccal mucosa is fast cellular recovery suggesting minimal damage to the administration site upon application (Reddy et al., 2011; Attia et al., 2004; Meng-Lund et al., 2014). The anatomical and physiological property of the buccal region leads to high drug bioavailability and fast onset of action. One such active which has been explored for buccal delivery is nitroglycerin. The API has been developed into various

sublingual dosage forms with notable advances in ointments, gels, film patches and tablets (e.g. low molecular weight heparins) (Radivojša Matanovic et al., 2015).

Several engineering methods have been developed to prepare buccal dosage forms which include solvent casting (Palem et al., 2010) and hot melt extrusion. Both methods exhibit advantages and limitations during formulation preparation. Electrospinning (ES) is an evolving and maturing engineering method with extensive recent interest in the pharmaceutical arena (Nazari et al., 2017; Mašek et al., 2017; Bhardwaj and Kundu, 2010). This one-step technique has been developed to fabricate fibrous films loaded or co-loaded with API (at required quantities *in-situ*) at the ambient environment (Mehta et al., 2017). Process aspects (applied voltage, flow rate and the distance between tip of processing nozzle to collecting platform) and pre-formulated material properties (e.g. viscosity, density, electric conductivity and surface tension) are often explored and correlated with processing viability (Reneker and Yarin, 2008; Jaeger et al., 1998; Tomczak, Van Hulst and Vancso, 2005). ES has numerous sister processes namely, electrospraying (Wu and Clark, 2007), EHD printing (J.C. Wang, H. Zheng, M.W. Chang, Z. Ahmad, J.S. Li, 2017), microbubbling (Ekemen et al., 2011) and these can be complexed further through the use of two or more processing needles (Rasekh et al., 2017).

NSAID drugs (Indomethacin, Ketoprofen, Diclofenac Sodium and Piroxicom) are non-steroidal anti-inflammatory drugs (hydrophobic) are available in crystalline form; which is used for the treatment of common illness such as fever, mild to moderate pain, swelling and to relieve symptoms of arthritis. NSAID drugs inhibit the production of prostaglandins which are responsible for inflammation and pain (Inada et al., 2013; Siddiqui et al., 2014). As a model drug, enhancement of NSAID solubility and dissolution at the buccal site requires amorphisation. This is crucial and enhances drug bioavailability as its increases molecular mobility when compared with the crystalline form (Alqurshi et al., 2016). Numerous studies have focused on the development of thin film platforms and the clear majority of these have utilised *in-vitro* or *ex-vivo* studies for evaluation, although greater bio-interface evaluation is needed. Furthermore, assessment using various models (*ex-vivo* and *in-vitro*) and how they correlate is valuable.

In the present work, amorphous fibrous of all four drugs (with similar co-polymer addition) films were prepared using Polyvinylpyrolidone (PVP) and Ethocel (E10) *via* single step ES. Films were characterised for their size and their components (presence and stability) using spectral techniques. Drug loading and encapsulation efficiency of the process was determined.

NSAID drugs state was also assessed using XRD analysis and the release behavior of the active was explored *in vitro* (PBS). PBS spreading behavior on engineered polymer films was evaluated using surface contact angle measurements.

4.2 Objectives

NSAID drugs are have different physical and chemical properties. Their solubility and dissolution rate are different, therefore, could influence the formulation and change the strength of intermolecular interaction among the formulations. The aim of this chapter is to assess each NSAID drugs in defined formulation from chapter 3 (slow and fast release) and therefore, compare the effect of each NSAID drug on the formulation. Furthermore, evaluate co-polymers in molecular interaction with new formulated NSAID drugs. This direct us to better understanding of NSAID drugs for intended use and preparation by electrospinning technique. As a result, evaluate the acceptable NSAID drug for intended use by electrospinning technique.

The resulting fibres were analysed by employing thermal analysis using differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA); spectroscopic analysis, Fourier transform infrared (FT-IR) was employed. The morphology of fibres was examined using Scanning Electron Microscopy (SEM). The sample preparation method was described in chapter 2.

4.3 Materials and methods

4.3.1 Materials

The information related to materials used in this chapter is in chapter 2 (method and materials)

Table 4. 1 Formulated fibres-sample compositions and their drug content.

Formulation		Polymer (5%w/v)	Drug (5%w/w)	Co-polymer (5%w/w)	Drug Content (%w/w)
Slow release	F1	Polyvinylpyrrolidone (PVP)	Diclofenac Sodium	Ethocel (E10)	4.86 ± 0.06
	F2	Polyvinylpyrrolidone (PVP)	Ketoprofen	Ethocel (E10)	3.4 ± 0.12
	F3	Polyvinylpyrrolidone (PVP)	Piroxicam	Ethocel (E10)	4.06 ± 0.0
	F4	Polyvinylpyrrolidone (PVP)	indomethacin	Ethocel (E10)	4.8 ± 0.05
Fast release	F5	Polyvinylpyrrolidone (PVP)	Diclofenac Sodium	-	4.68 ± 0.08
	F6	Polyvinylpyrrolidone (PVP)	Ketoprofen	-	3.96 ± 0.07
	F7	Polyvinylpyrrolidone (PVP)	Piroxicam	-	2.43 ± 0.05
	F8	Polyvinylpyrrolidone (PVP)	indomethacin	-	4.98 ± 0.01

4.3.2 Methods

Fibres preparation method is as same as the method mentioned in chapter 2 (methods and materials). All preparations contained PVP (5% w/v) and NSAID drugs (5% w/w of PVP). Other excipients were added in quantities shown above as a function of w/w % of PVP.

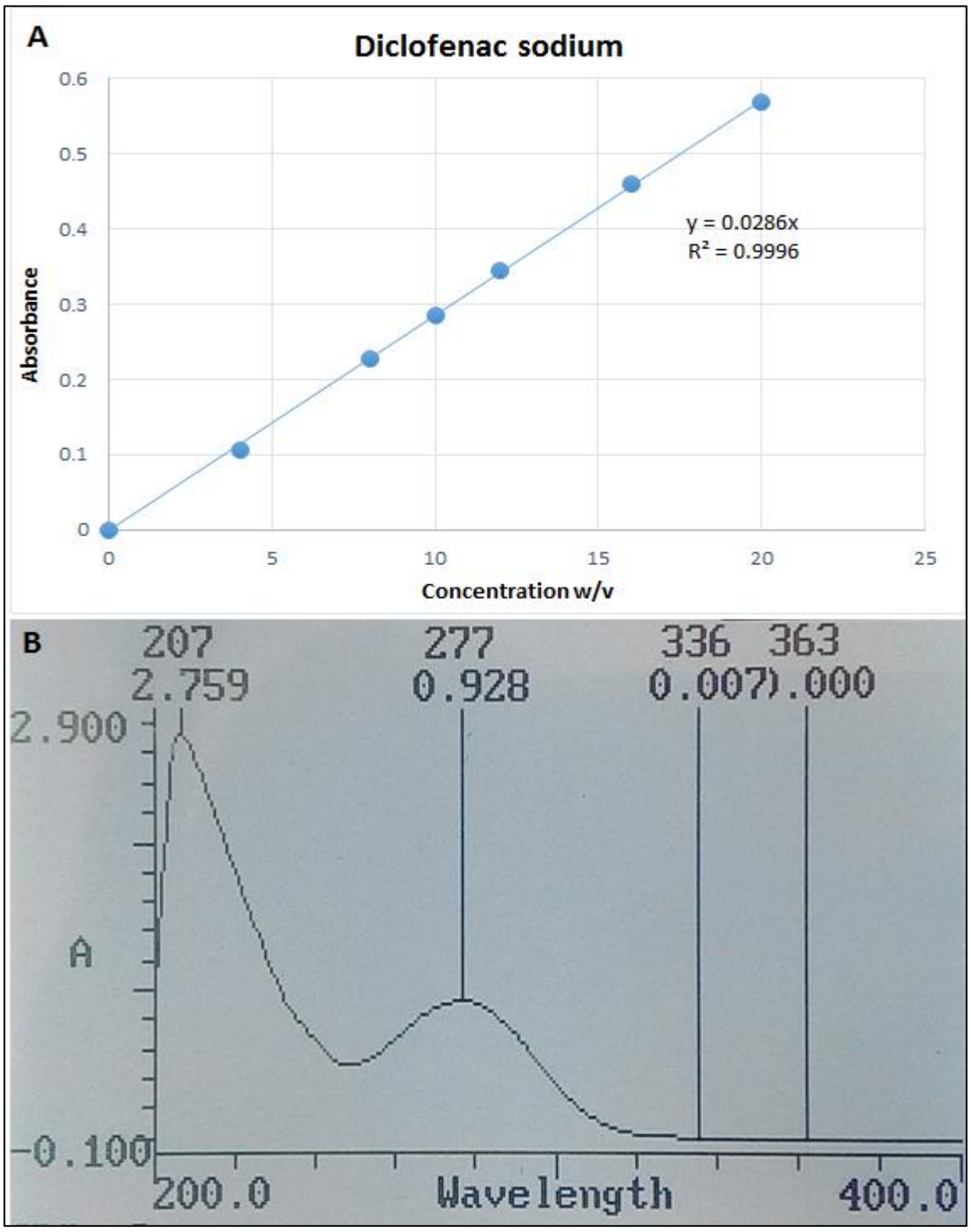


Figure 4. 1 (A) UV-Vis calibration curve (B) UV-Vis spectrometer beam of Diclofenac sodium in PBS at ambient temperature

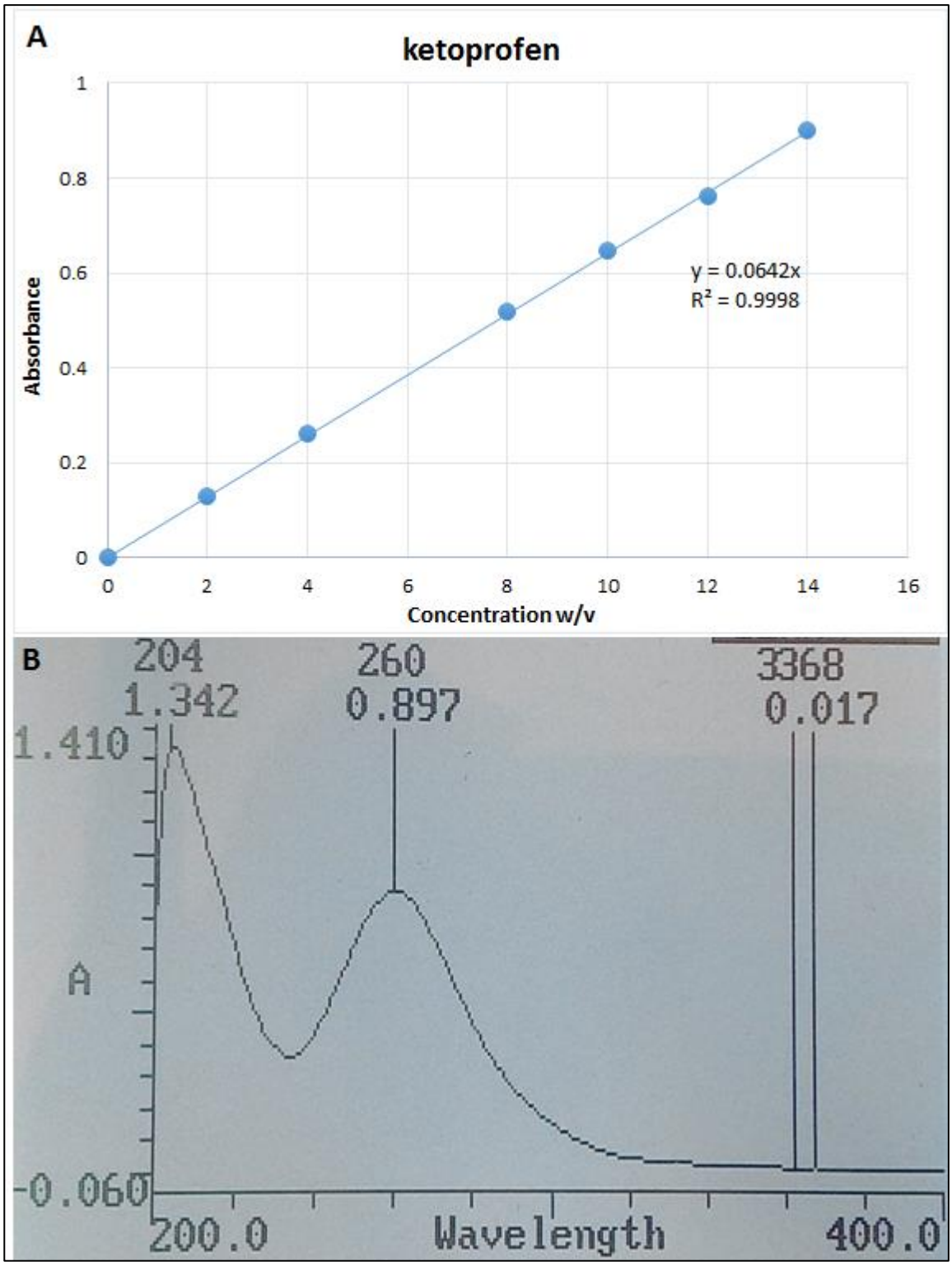


Figure 4. 2 (A) UV-Vis calibration curve (B) UV-Vis spectrometer beam of Ketoprofen in PBS at ambient temperature

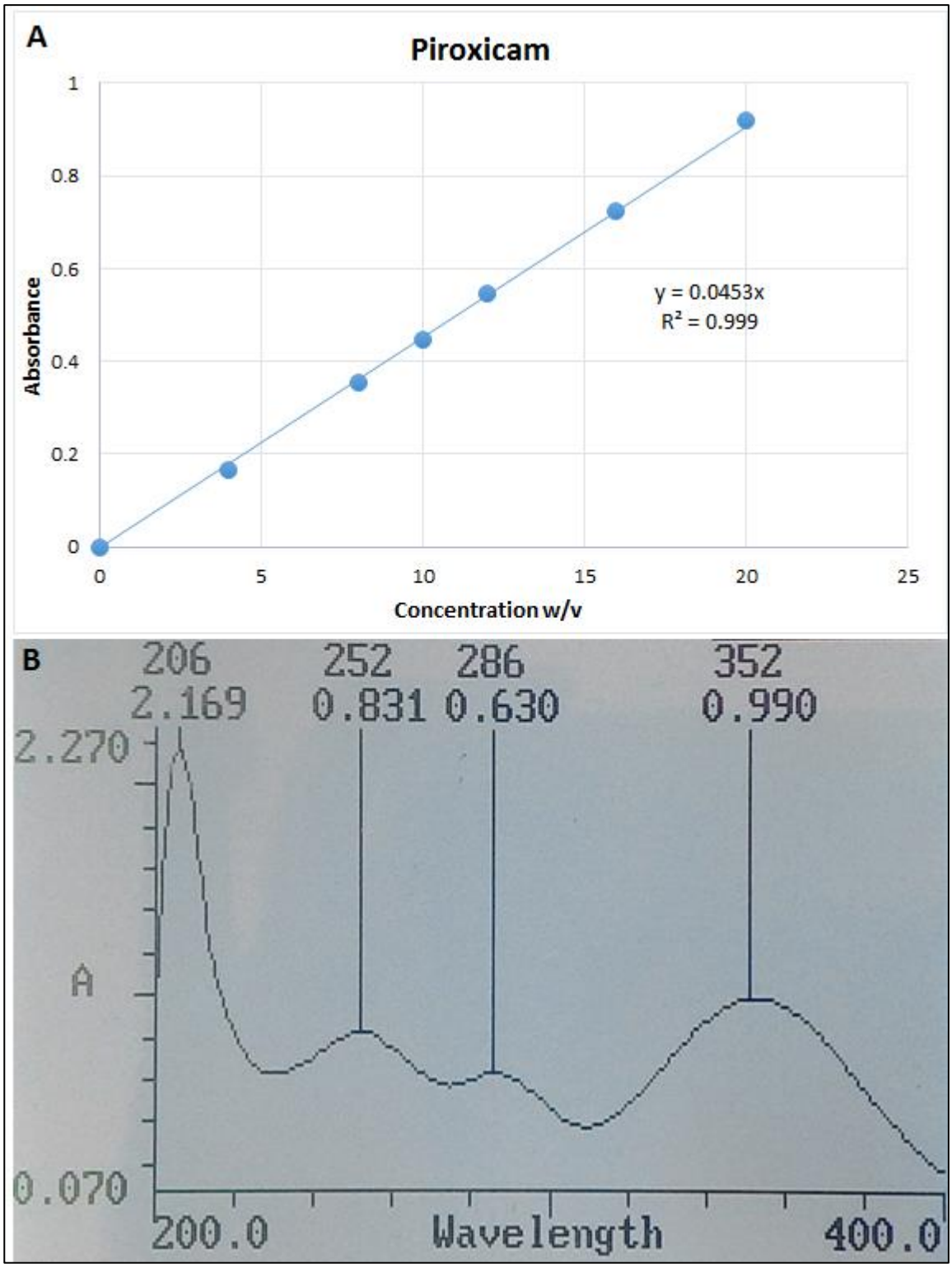


Figure 4. 3 (A) UV-Vis calibration curve (B) UV-Vis spectrometer beam of Piroxicam in PBS at ambient temperature

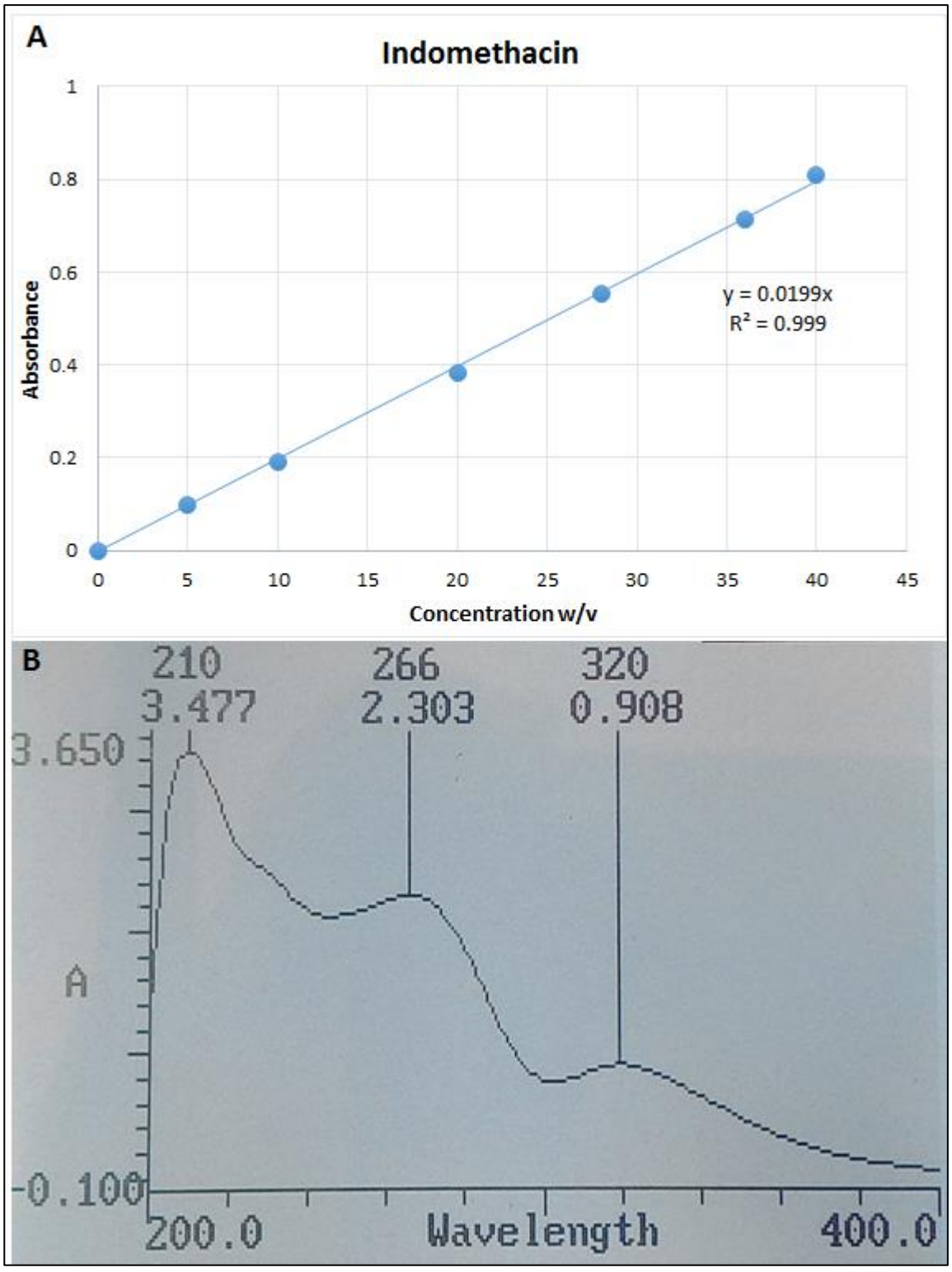
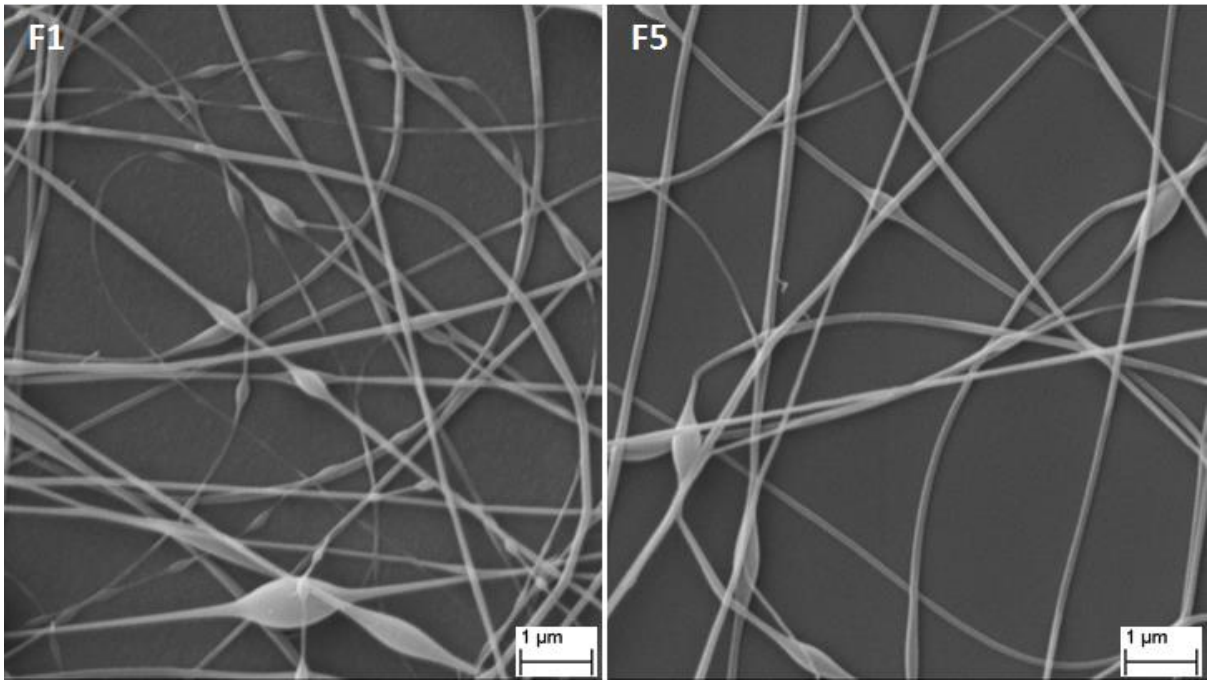


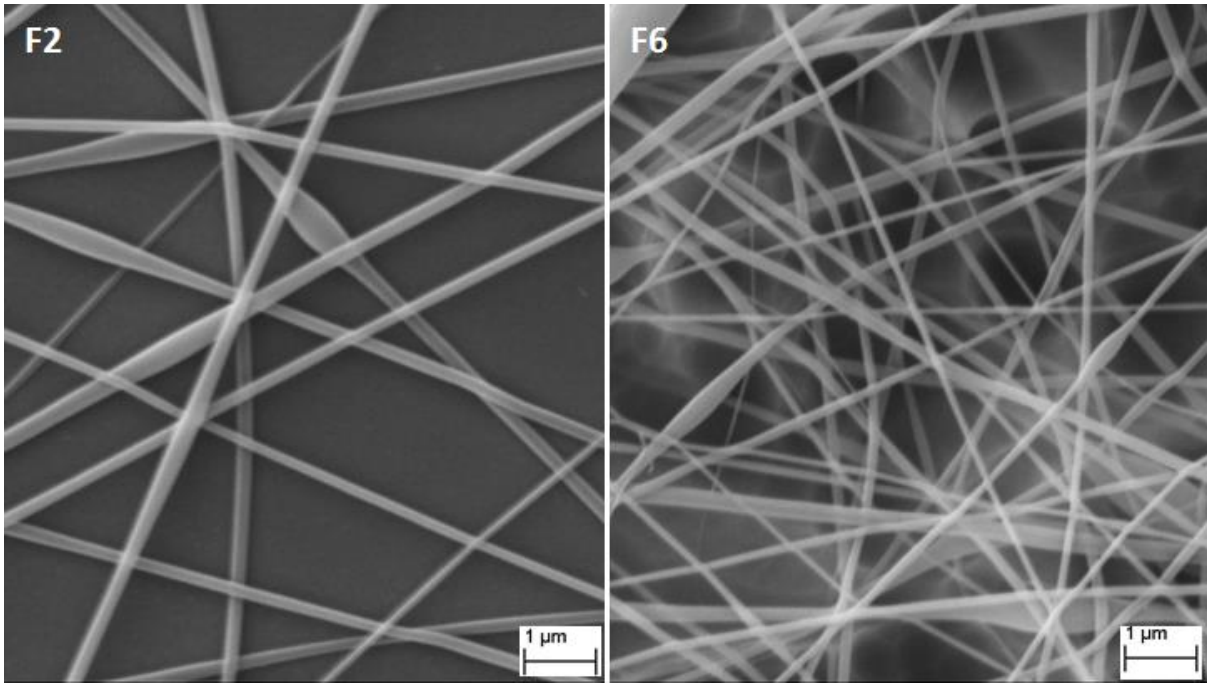
Figure 4. 4 (A) UV-Vis calibration curve (B) UV-Vis spectrometer beam of indomethacin in PBS at ambient temperature.

4.4 Results and discussions

Fibrous membranes were engineered using all different NSAID drugs (solution) formulations (4 slow release and 4 fast release). Stable jetting was determined using a preliminary applied voltage contrasted with infusion rate experimental matrix to ensure near uniform fibre engineering. To achieve this stable jetting, several modes are encountered which become apparent for various types of media (Arumuganathar, Jayasinghe and Suter, 2007; Jaworek et al., 2004) at the nozzle exit. In addition, various jetting modes have been developed based on material properties and needle alignment and these have an impact on the end structure (Nangrejo et al., 2008; Zhang et al., 2015). However, stable jetting windows were determined for NSAID-PVP based formulations and were subsequently used to engineer fibrous films.

Figure 4. 5 show surface morphology of various electrospun fibrous membranes with inset images displaying membrane width (thickness), obtained using plan view microscopic analysis. All formulated fibres exhibit a smooth surface; albeit with slightly variable diameter distributions. The difference is attributed to the presence of additional co-polymer in the formulation (Nazari et al., 2017). Figure 4. 6 shows diameter distributions of 250 randomly selected fibres from each electrospun membrane sample. The fibre diameter was noted to slightly reduce for most of the formulation upon the inclusion of co-polymer (F6, F7 and F8). All formulation displays mean fibre diameters of less than 500 nm. However, formulation which cooperated with copolymer not only shows lower fibre dimeter size but also better morphology. (Nazari et al., 2017; El-Naggar et al., 2016).


F1
1 µm
F5
1 µm


F2
1 µm
F6
1 µm

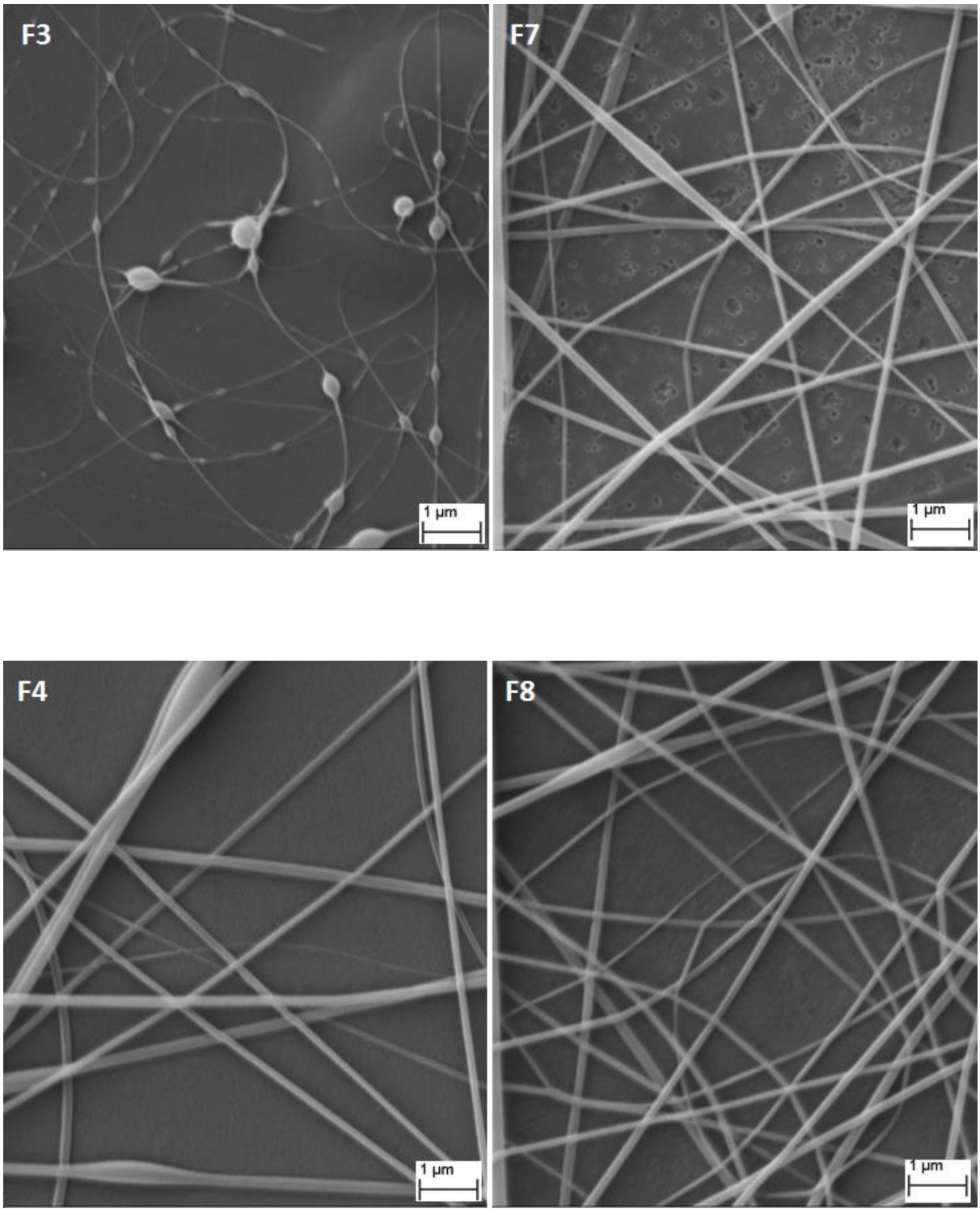
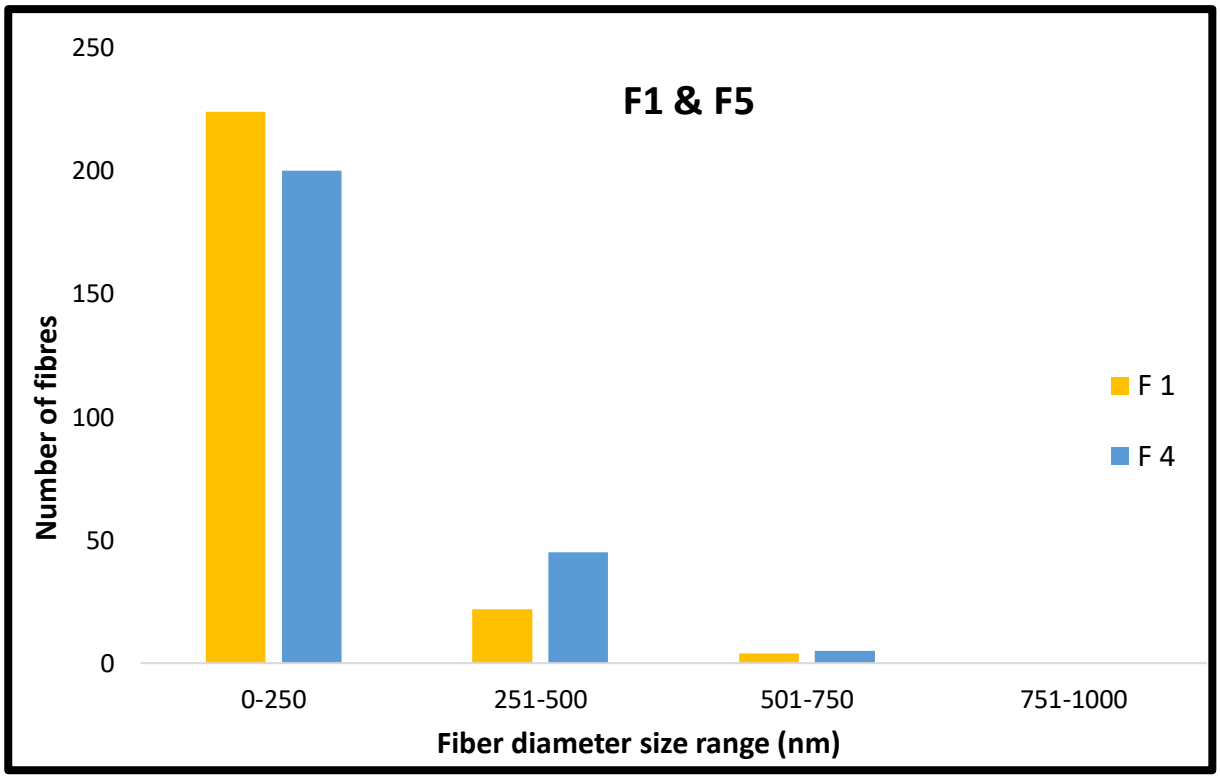
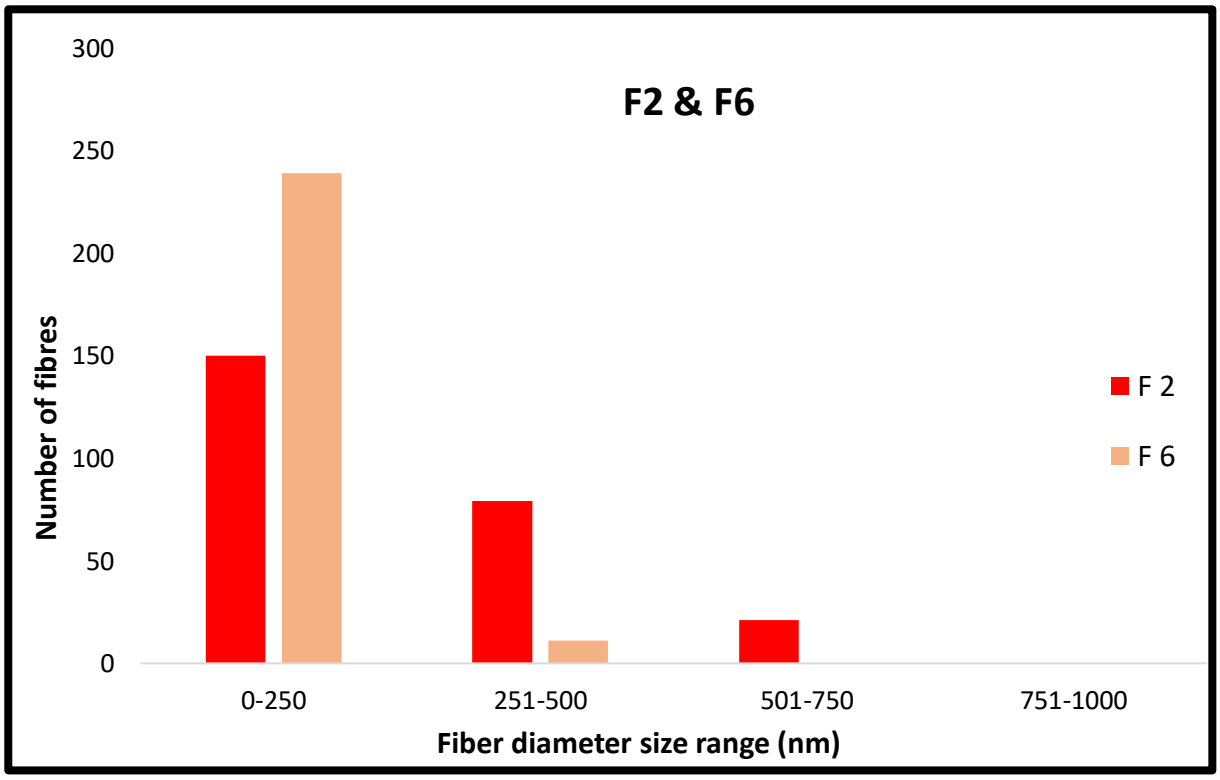
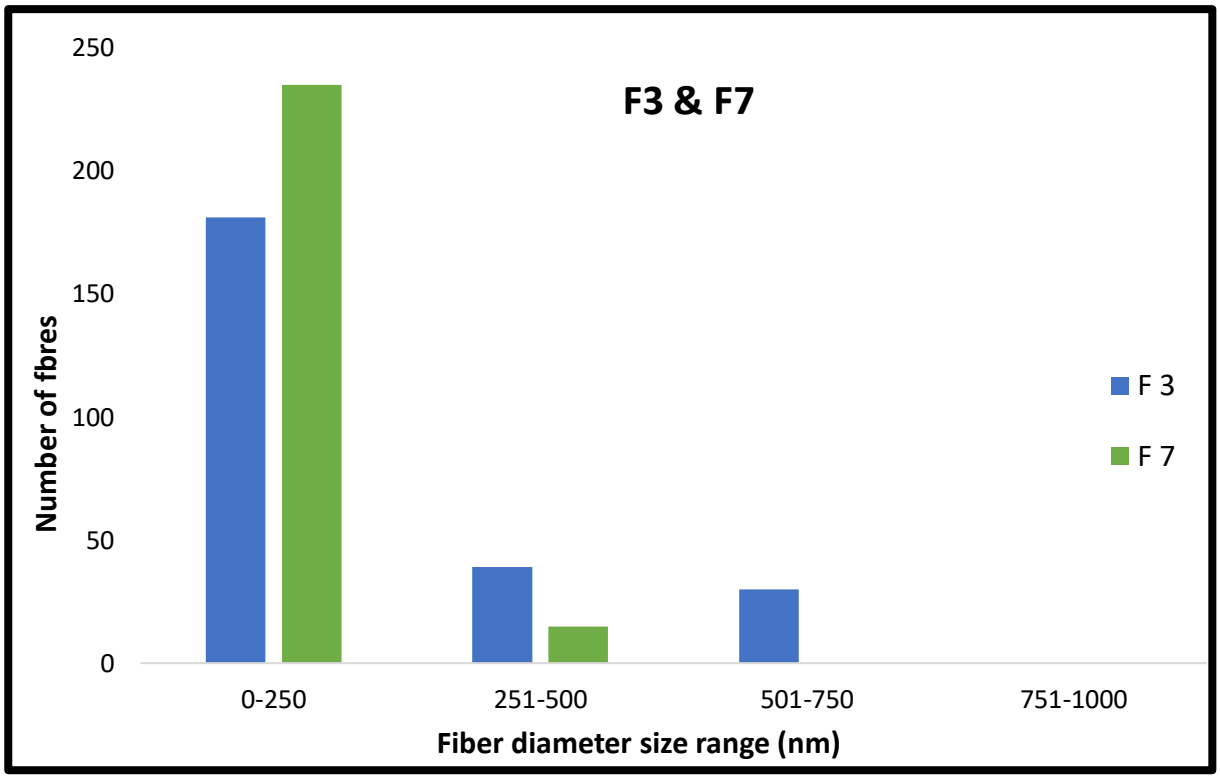


Figure 4. 5 Electron microscope of films samples. Comparing fast and slow release morphology for each active pharmaceutical ingredient (Slow release; F1 -F4 and fast release; F5 – F8)


F1 & F5
250
200
150
100
50
0
Number of fibres
0-250
251-500
501-750
751-1000
Fiber diameter size range (nm)
F 1
F 4


F2 & F6
300
250
200
150
100
50
0
Number of fibres
0-250
251-500
501-750
751-1000
Fiber diameter size range (nm)
F 2
F 6



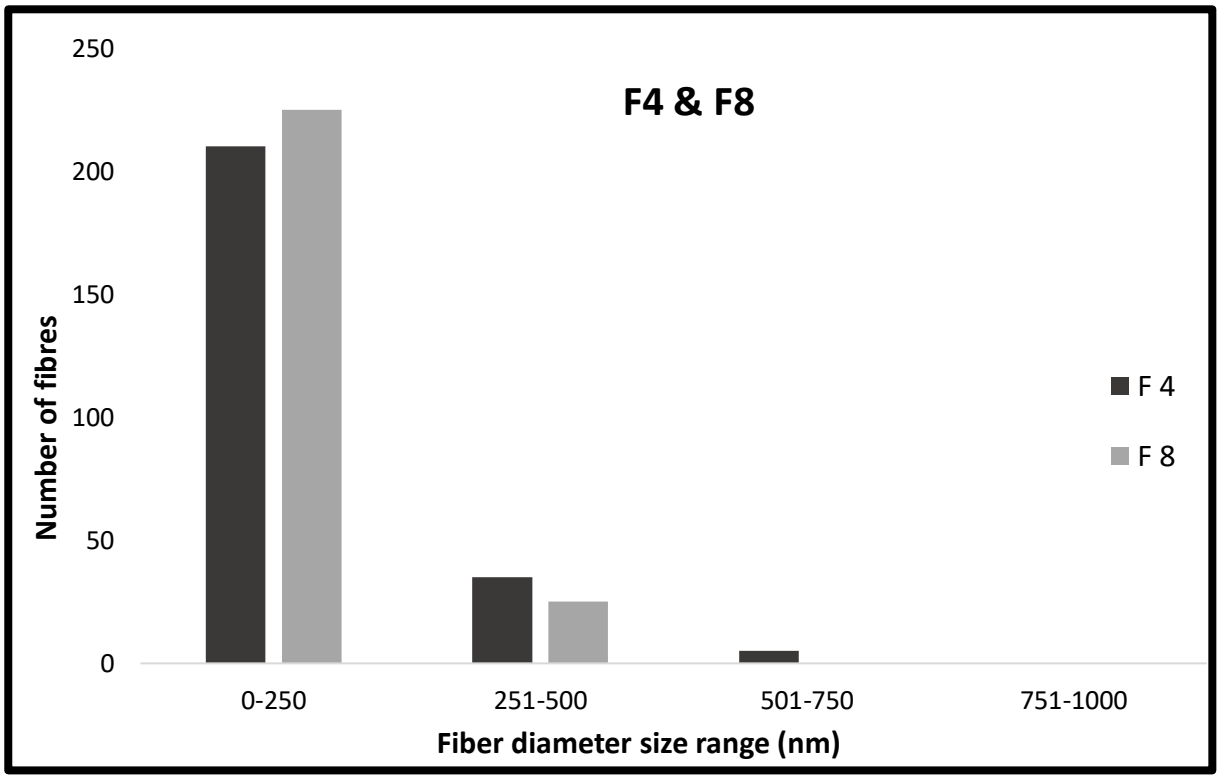


Figure 4. 6 Fibre diameter size distribution of formulated films. (Comparing both slow and fast release formulation for each API).

XRD patterns of electrospun fibrous films and neat NSAID drugs are shown in Figure 4. 7. The X-ray diffraction pattern of pristine NSAIDs indicate the active possess a highly crystalline structure by means of multiple sharp diffraction peaks at (∼2 theta = 10–38°) and principle peaks at 11.7, 16.7, 19.5, and 21.6 were observed for indomethacin (Nazari et al., 2017). The XRD profile of diclofenac sodium indicate the crystalline nature of this drug as sharp peaks can be observed at 11.1, 15.1, and 27.6. Sharp peaks at 5.5, 18.3, and 22.7 in ketoprofen XRD profile also indicate the drug crystallinity. Piroxicam XRD profile shows sharp peaks at 8.5, 14.2, 17.4, and 27.1 which illustrate the crystalline nature of the drug.

The diffraction spectrum of all electrospun fibrous films (F1–F8) show a diffused background pattern with complete disappearance of all principle NSAID drugs peaks. This suggests NSAID drugs are well dispersed in fibrous matrices, yielding an amorphous state. The amorphous state provides several benefits, including accelerated drug release and enhanced drug solubility, which are valuable for bioavailability. However, this must be correlated with permeation behavior to gauge real benefit.

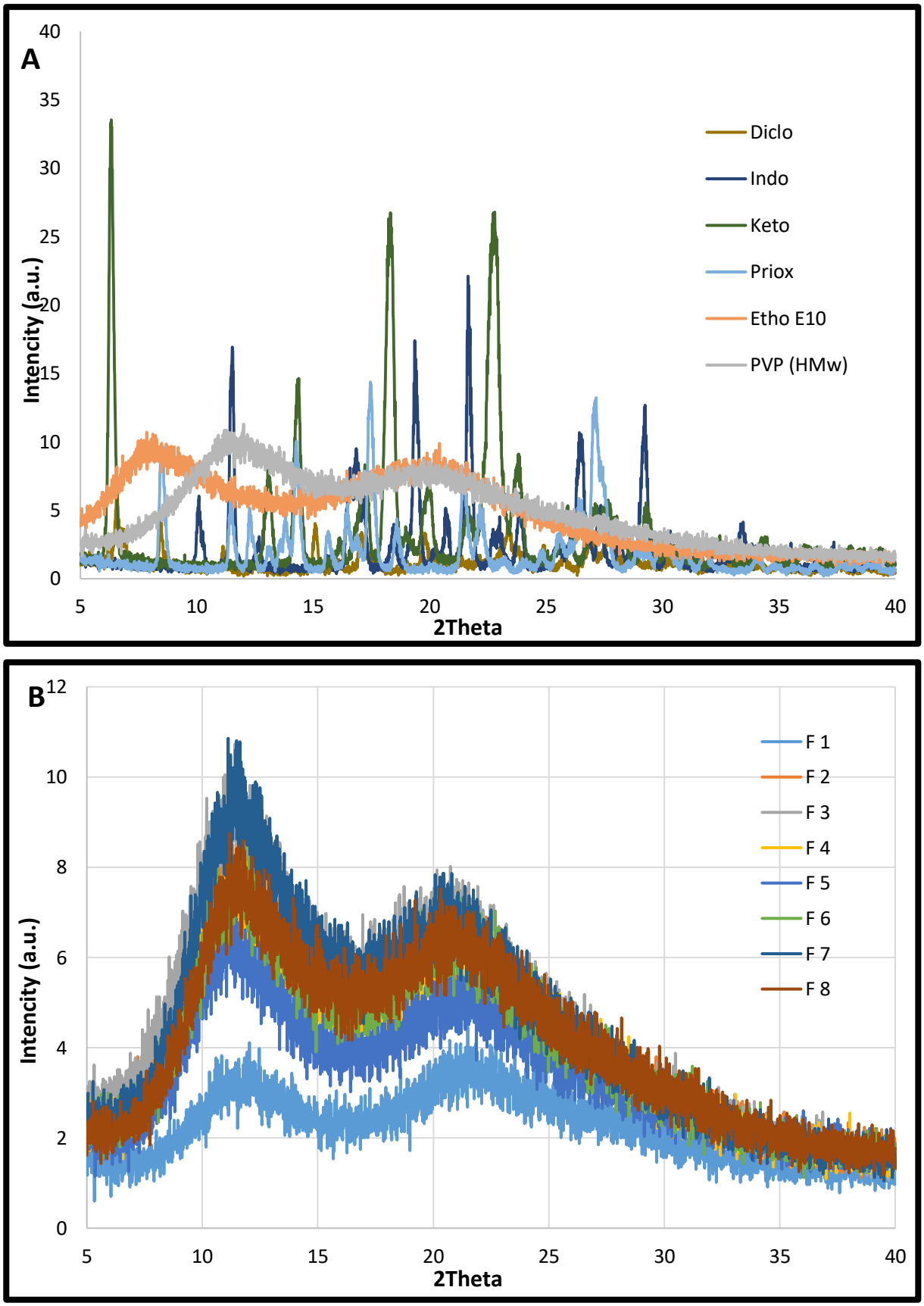


Figure 4. 7 X-ray diffractograms of (A) raw material and API, (B) formulated film fibres.

Figure 4. 8 and Figure 4. 9show DSC and TGA thermograms of electrospun NSAID formulations. In its obtained form, NSAID drugs are crystalline and possesses a melting temperature (T_m) of ~ 295,100, 209, and 164 °C for Diclofenac sodium, ketoprofen, piroxicam, and indomethacin respectively (as shown in Figure 4. 8A). This melting peak is no longer observed for electrospun samples, indicating the drug is dispersed throughout the polymer matrix post ES. Numerous studies have made use of the ES technique to engineer PVP fibres from the raw polymer (Yu et al., 2010; Kamble et al., 2016; Li et al., 2012). In these, a broad endotherm arising at ~80 °C has been reported which also the case in this study for most of the samples which is prepared using NSAID and PVP. The broad endotherm at this value is attributed to dehydration which is most likely due to loss of moisture often adsorbed by hygroscopic PVP polymer, the rate of which may be enhanced due to the increased surface area of fibrous structures. Furthermore, cooperation of the ES co-polymer formulation content provides greater polymeric matrix volume for active spacing and dispersion.

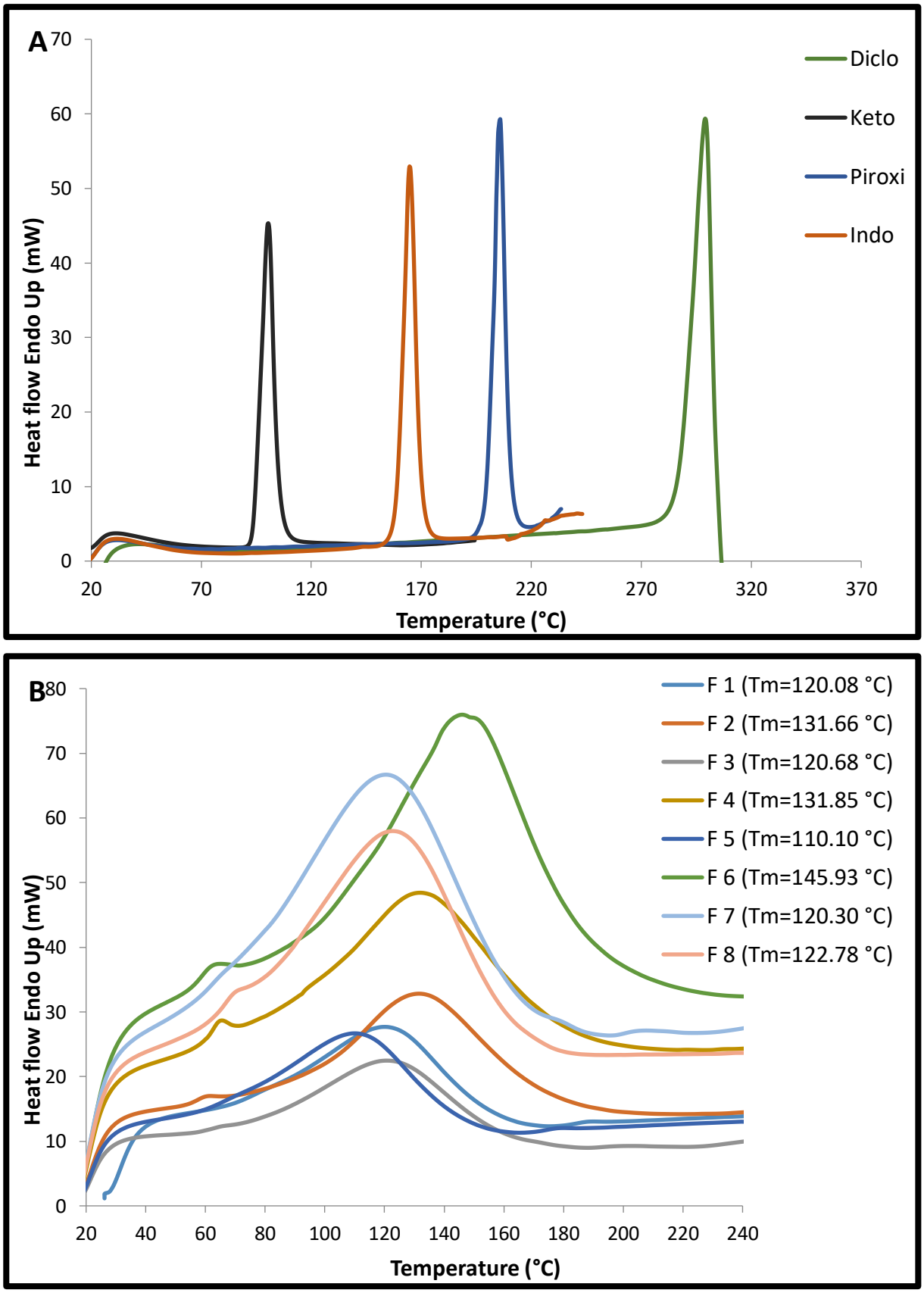


Figure 4. 8 Thermal analyses of A) selected APIs and B) engineered films using differential scanning calorimetry.

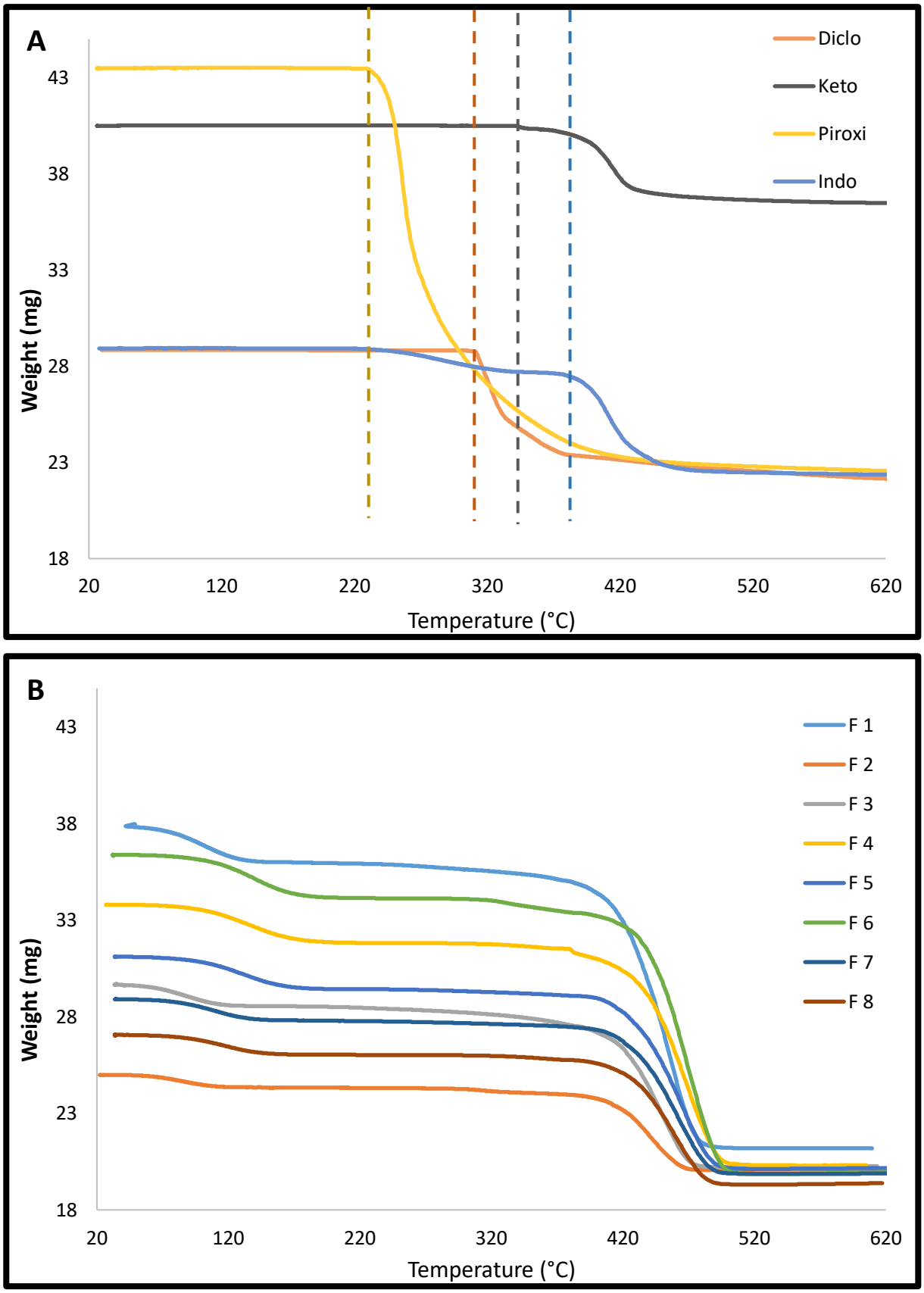


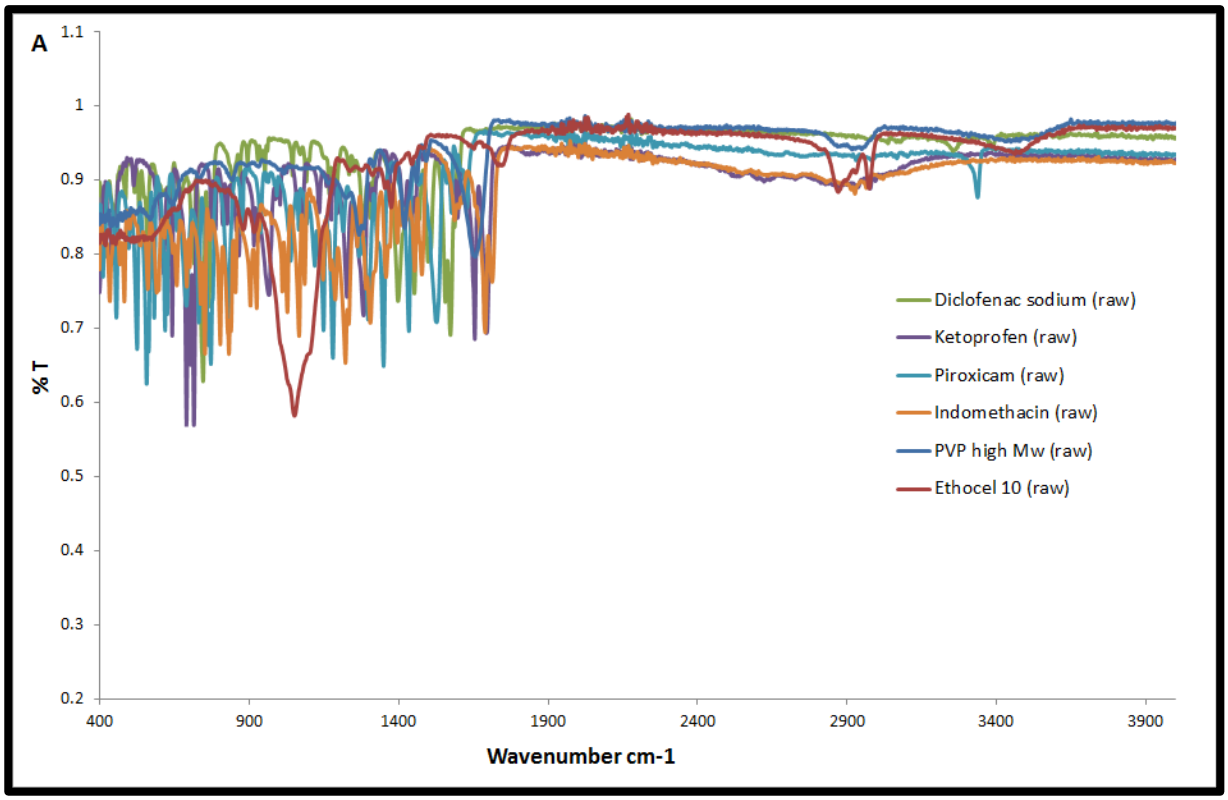
Figure 4. 9 Thermal analyses of A) selected APIs and B) engineered films using Thermal gravimetric analysis.

TGA was utilized to study thermal degradation behavior of fibrous NSAID-polymer composite films. Figure 4. 9 A and B show TGA thermograms of electrospun formulations and raw NSAID drugs. In the current study, all fibrous films show two degradation steps (Figure 4. 9B); the first relates to weight loss in film formulation below 100 °C, which is ascribed to evaporation of residual solvent (ethanol: water) or surface adsorbed moisture on fibrous (and porous) samples. The second weight loss step occurs in the range 350–500 °C; and is attributed to degradation and decomposition of components comprising fibrous formulations such as PVP and co-polymer excipients. All C—H, C—N, C═O and C—C bonds are compromised with temperatures exceeding 300 °C; after which complete decomposition of PVP into nitrogen and carbon oxide powders is observed (Shi, Lu and Jiang, 2009). Previous studies indicate similar degradation steps (Taepaiboon, Rungsardthong and Supaphol, 2006).

TGA thermogram of PVP-NSAID drugs formulations (F1, F3, and F4) shows the highest temperature associated with the second weight-loss step when compared with same formulation with cooperation of co polymer. On the other hand, formulations F2 and F6 show opposite, as same formulation with added copolymer increase the weight-loss step.

This indicates that incorporation of a co-polymer excipient into the fibrous drug loaded film has different effect on the degradation of prepared fibre in respect to NSAID drug. NSAIDs are loaded directly into filamentous matrices (Nazari et al., 2017) and previous studies report that a shift in the maximum temperature (associated weight-loss step) can be used to validate drug entrapment within films (Taepaiboon, Rungsardthong and Supaphol, 2006). The thermal degradation of a composite formulation is also driven by the active ingredient (drug) used; as both increase and decrease in the maximum temperature associated weight loss have been shown (Taepaiboon, Rungsardthong and Supaphol, 2006).

FTIR spectroscopy was used to study structure and molecular conformation of fibrous films by probing band vibrations (Baghel, Cathcart and O'Reilly, 2016). Prepared fibres (PVP-NSAID drug) which could be attributed to increased amorphous NSAID drug content. The data suggests active drug, base polymer (PVP) and co-polymer (Ethecol™ E10 (where integrated) incorporate completely into filamentous structure by ES.



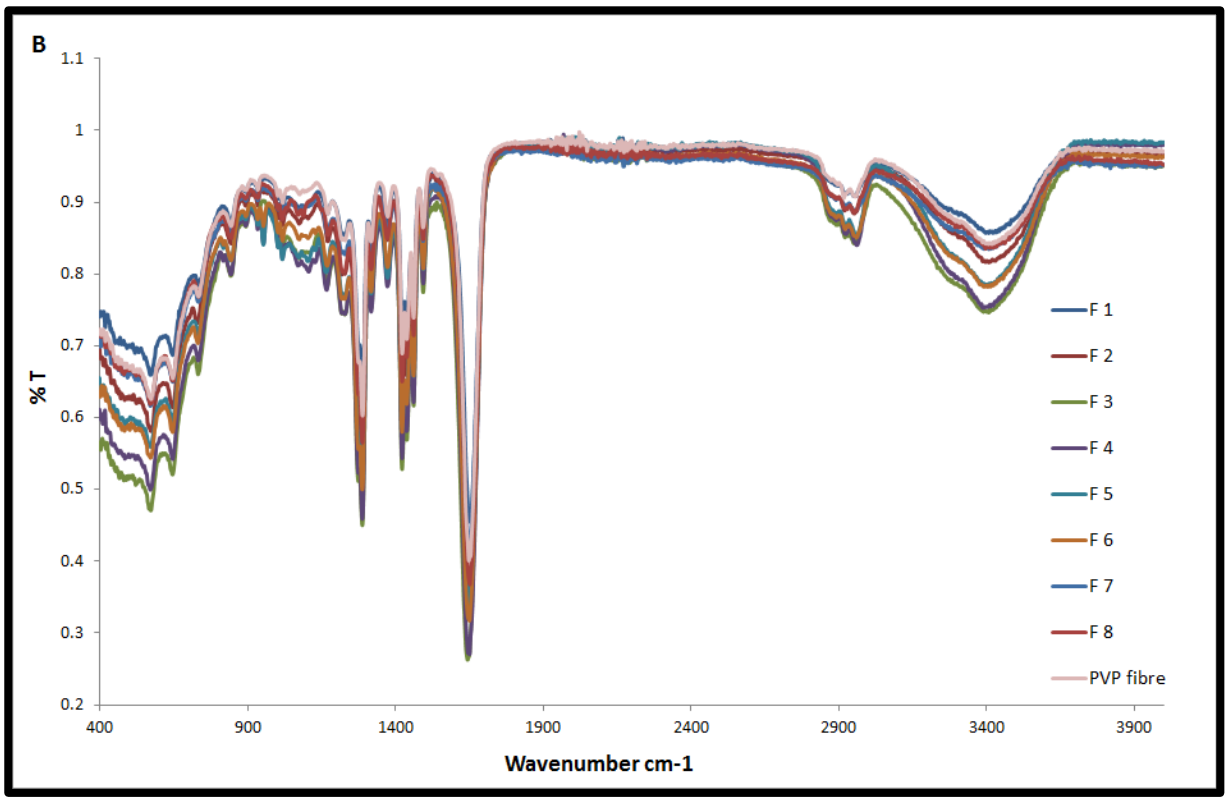
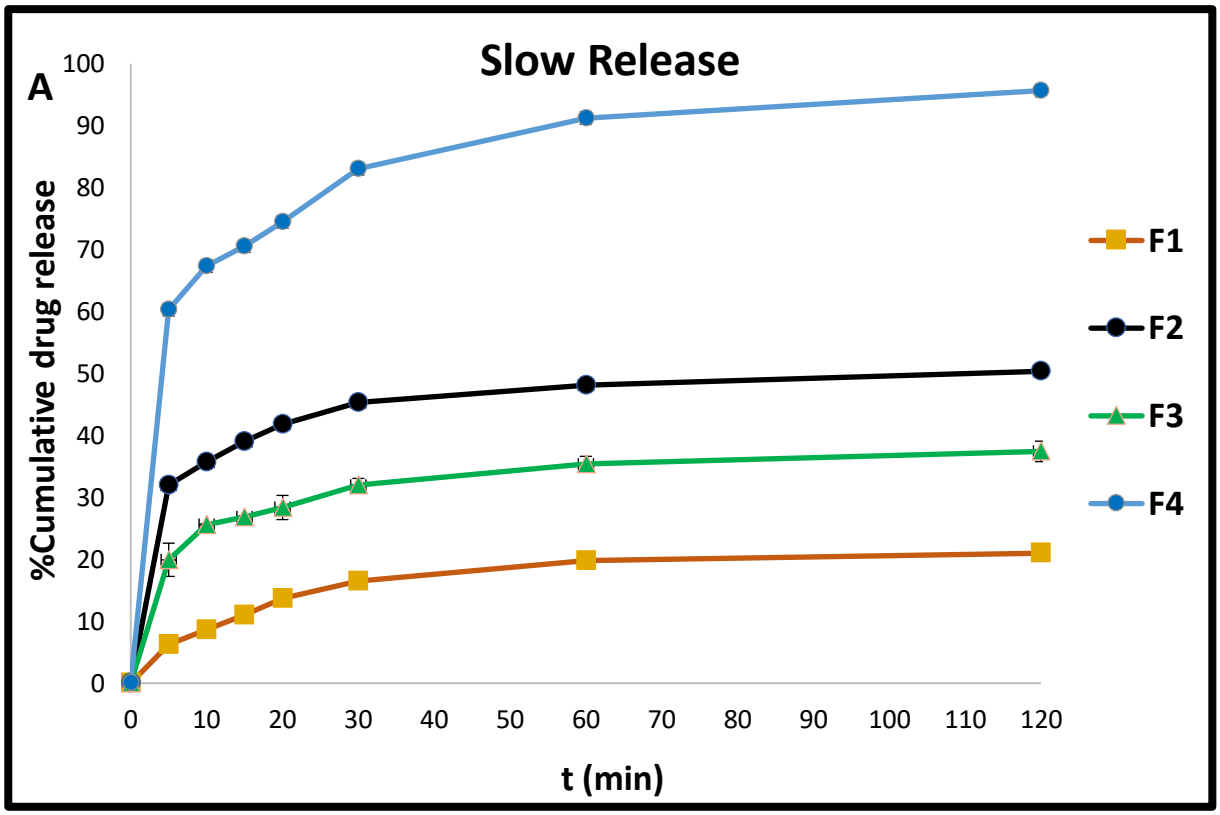


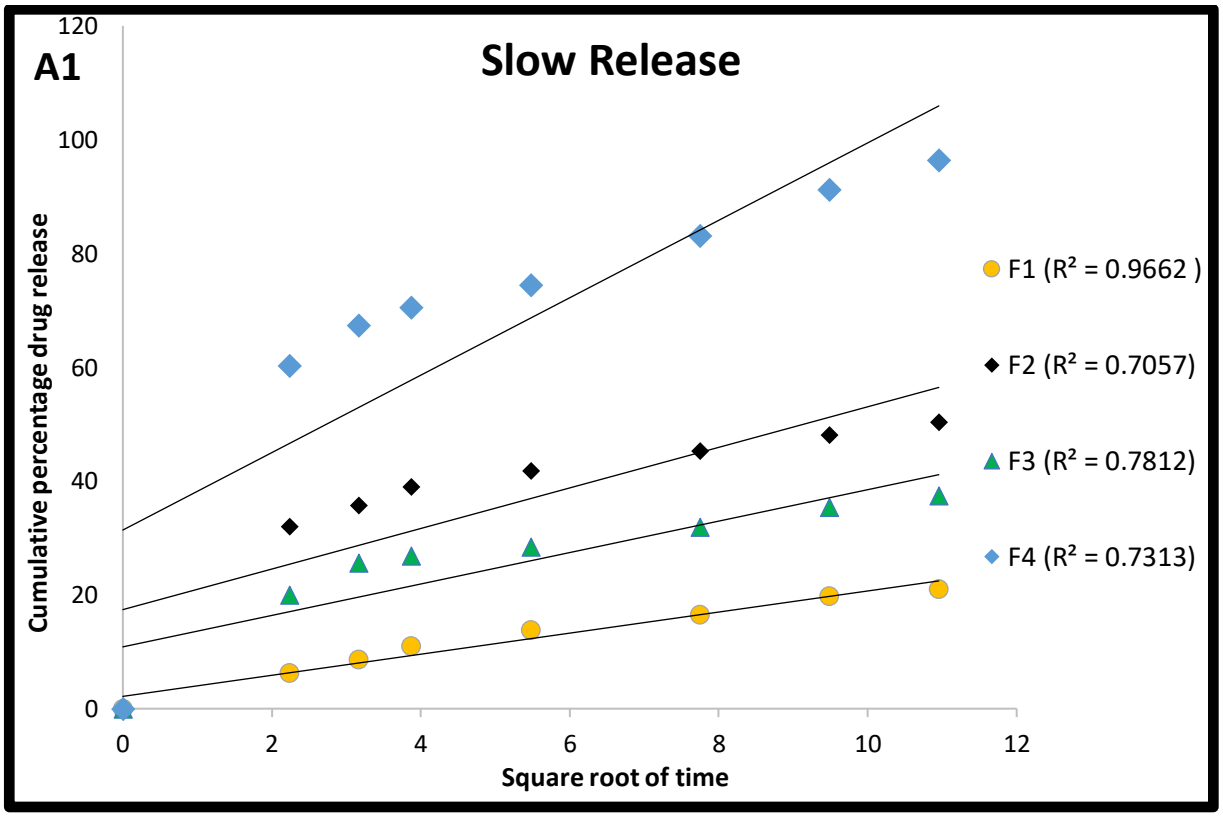
Figure 4. 10 Spectroscopic analysis of engineered films and selected excipients using Fourier transform infra-red.

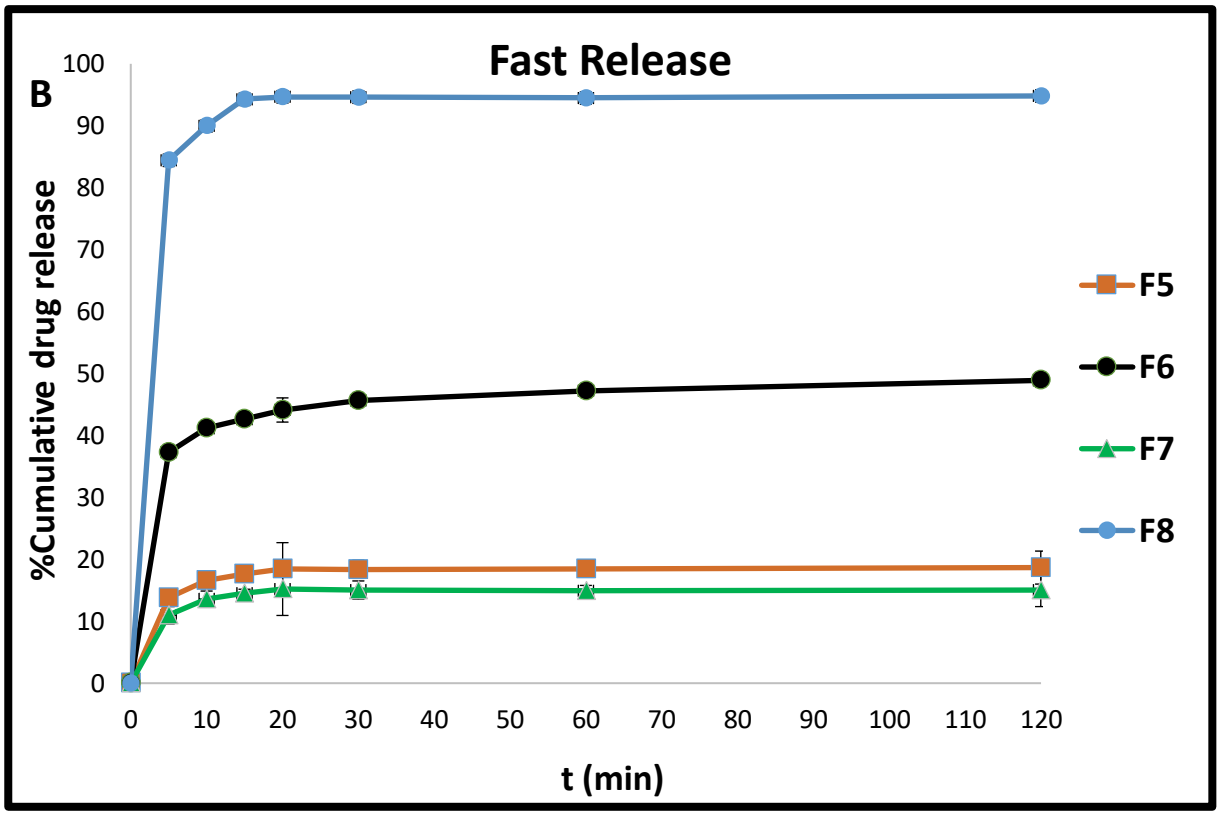
Figure 4. 10 A and B show FTIR spectra at the wavelength range 4000–400 cm^{-1}. PVP background is observed in all samples and various characteristic peaks for other excipients and the active are detected. Observed peaks for Indomethacin were amide C═O at 1600 cm^{-1} which is due to vibrations arising from aromatic ring. In addition, absorption peaks are observed at 2980 and 1423 cm^{-1} (attributed to C—H vibration of methyl and methylene) (Shi, Lu and Jiang, 2009) and C═O vibration at 1680 cm^{-1} from PVP polymer (peaks display weaker intensities at ~1661 cm^{-1}) due to hydrogen bonding with the active and other co-polymers (Borodko et al., 2006). The spectrum of Piroxicam shows absorption bands due to C═O at 1112 cm^{-1} and OH at 3300-3500 cm^{-1} stretching (Lai et al., 2011). The spectrum of diclofenac sodium shows absorption of 3385 cm^{-1}, 1600 cm^{-1} and 1555 cm^{-1} due to NH (secondary amine) C═O and C═C stretching respectively (Das and Subuddhi, 2015). The spectrum of Ketoprofen shows absorption at 1690 and 1655 which attributes to the C═O stretching of carbonyl group (Chan et al., 2015). The spectrum exhibits identical for all formulation with slight changes in peak intensity due to presence of copolymer in some formulations. This is because of Van der Waals forces of attraction or hydrogen bonding in formulation contain copolymer. New peak is observed between 1087–1113 cm^{-1}, and is ascribed to carboxylic acid carbonyl stretching within copolymer.

Table 4. 1 represents drug content in fibrous films which is ranged from 2.43 to 4.98% w/w. The values are less than the theoretical maxim (5% w/w) incorporated into ES formulations prior to processing. This is attributable to drug precipitation during the ES process or shortly thereafter. Moreover, sample prepared using copolymer (Ethocel E10) in cooperation with Piroxicam reveals greater drug encapsulation compared to formulation without copolymer. Other formulations, with adding copolymer (Ethocel E10) the encapsulation show slightly reduction.

Cumulative release profiles of various NSAID formulations are illustrated in Figure 4. 11. The presence of Ethocel E10 results in a decrease in API release rate (formulations F1,F2, F3, and F4) compared to the single polymer-drug system PVP-NSAID formulations (F5, F6, F7, and F8).


A
Slow Release
%Cumulative drug release
t (min)
F1
F2
F3
F4


A1
Slow Release
Cumulative percentage drug release
Square root of time
F1 (R² = 0.9662)
F2 (R² = 0.7057)
F3 (R² = 0.7812)
F4 (R² = 0.7313)



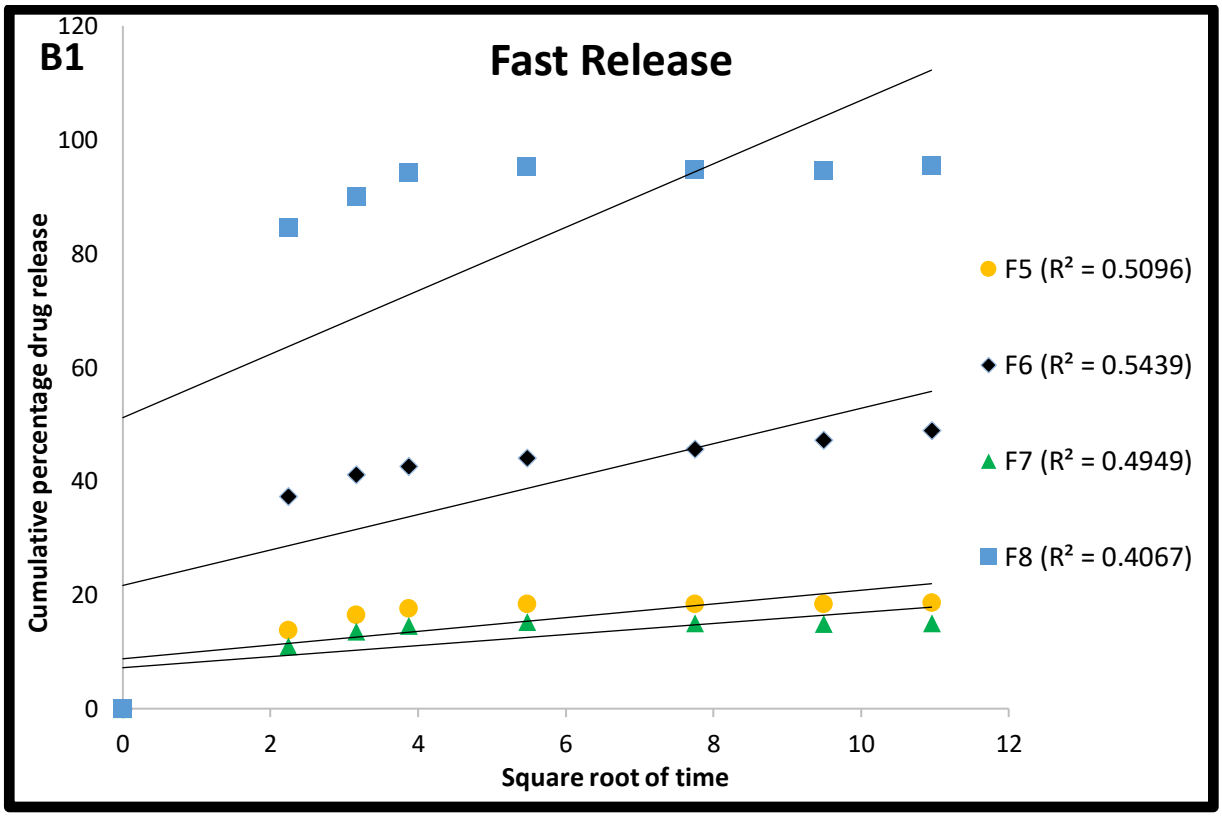


Figure 4. 11 Release profiles of A) F1, F2, F3, and F4 (slow release), and B) F5, F6, F7 and F8 (fast release) formulations in buffer pH 6.8. A1 and B1 show plots of cumulative release against root time for corresponding formulation.

A reduction in the release rate of NSAID drugs were observed by adding water insoluble polymer (Ethocel (E10)) (F1, F2, F3, and F4) in the formulated systems Figure 4. 11A. The effect of ethyl-cellulose content on drug release from polymer beads containing NSAID drugs has been shown, that with adding co-polymer the release rate has reduced. This is explained due to the non-soluble nature of the material which limits water interaction with the polymeric matrix.

Slow release formulations group (F1, F2, F3, and F4) show a steep and fast release within first 5 mins followed by a steady increase up to 12% for F1, 43% for F2, 29% for F3, and 91% for F4. NSAID drugs show different characteristic and therefore each present different release profile.

Fast release formulations group (F5, F6, F7, and F8) show a sharp and fast release within the first 5 min followed by a steady increase. However, the release rate was different for each formulation. F8 shows the highest release rate and with a steady rate up to 91%. F5 and F7 show the lowest release at about 12%. F6 shows a steep and fast followed by a steady release up to 43%.

The kinetic release model of PVP/NSAID/co-polymer nanofibers is shown in Figure 4. 11A1 and B1, in which the cumulative quantity of drug released per square centimeter of fibrous films is plotted against time and fitted to the Higuchi model. Drug release from all formulations showed two distinct release phases; an initial burst type release phase followed by a lag period. It was observed that the release profile of NSAID drugs from fibrous samples of F1 ($R^2 = 0.9662$), F2 ($R^2 = 0.7057$), F3 ($R^2 = 0.7812$), F4 ($R^2 = 0.7313$), F5 ($R^2 = 0.5096$), F6 ($R^2 = 0.5439$), F7 ($R^2 = 0.4949$), and F8 ($R^2 = 0.4067$) suggest that the release of the drug in this formulation is governed by diffusive mechanisms. This implies NSAID release is based on Fickian diffusion, based on drug permeation through the fibrous polymeric network.

Higuchi kinetic model fitting was applied to the initial phase. All slow release formulations show high linearity with R^2 (>0.7). It can be concluded that the dominating mechanism for NSAID drug release is Fickian diffusion. However, the release rate is different for each formulation; where F possessed the greatest K value and F5 possessed the lowest K value.

4.5 Conclusion

In the current study an array of formulated films containing NSAID drugs were fabricated using electrospinning ES technology. The nanofibers showed consistent fibre morphology, which would improve the release of drug from matrix to the skin due to large surface area. However, some of the electrospun formulations showed beading within the nanofibres, and therefore the formulation requires further optimizations for each of APIs and incorporated polymer/co-polymer.

The drug associated with the fibres was in an amorphous state (molecularly dispersed) as evidenced from the X-ray studies whereas the release rate was mainly controlled by polymer composition in a sustained manner. The *in vitro* release of NSAID were regulated by the quantity and the hydrophilicity/hydrophobicity ethyl-cellulose in the formulation. The strategy proposed in this study provides a method to design novel NSAID-polymer hybrid electrospun mats utilized for buccal delivery.

By using this technique, it has been proved that NSAID drugs can be encapsulated in polymeric nanofibres matrix in order to achieve the amorphous state of drug for buccal drug delivery.

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Chapter 5 Comparing Electrospinning films with Casting films

5.1 Introduction

Pharmaceutical industry has put a large amount of efforts into the actives that work on new technologies, to tackle problems associated with drug administration and drug delivery system which is effective stage to progress pharmaceutical and therapeutics of drug. These systems help drugs to overcome barriers which are associated with actives, such as low solubility due to the nature of drug hydrophobicity, and therefore prevent degradation of drug prior to reach the target area (Santos et al., 2018; Allen and Cullis, 2004). Many systems and devises have attracted attention for oral cavity application and absorption as oral cavity provide a large surface area for drug absorption (Prausnitz, Mitragotri and Langer, 2004). Transdermal delivery developed successfully in past years and has gained approval for many drugs (Santos et al., 2018; Wiedersberg and Guy, 2014). It is a reliable alternative to the oral route, both for local and for systemic delivery of drugs, overcoming some of the drawbacks existent in oral administration, avoids the first-pass effect, pre-systemic elimination by the gastrointestinal tract, adverse drug reactions and moreover, provide a fast absorption of drug (Santos et al., 2018; Naik, Kalia and Guy, 2000). The buccal route for drug administration has gained importance in the past years due to its accessibility and rapid onset of action. It is a reliable alternative to the oral route, both for local and for systemic delivery of drugs.

The buccal route of administration is a specific type of drug delivery that involves the patient placing the drug in the gap between the gum and the cheek until it completely dissolves. During this time, the active pharmaceutical ingredient (API) will penetrate from the drug formulation into the bloodstream through the tissue lining of the mouth, or oral mucosa, via diffusion.

The buccal route enhances absorption of poorly absorbable drug and providing an effective drug delivery (Sudhakar, Kuotsu and Bandyopadhyay, 2006; Al-Dhubiab et al., 2015). Buccal delivery system attracted many scientists because can be developed in such as a reservoir for sustain release systems (Al-Dhubiab et al., 2015; Downing et al., 2014).

Many methods have been developed to prepare buccal dosage forms which include solvent casting (Palem et al., 2010) and hot melt extrusion (HME) (Morales and McConville, 2011). Currently under research however, is the formulation of buccal films by making drug-loaded fibres via electrospinning.

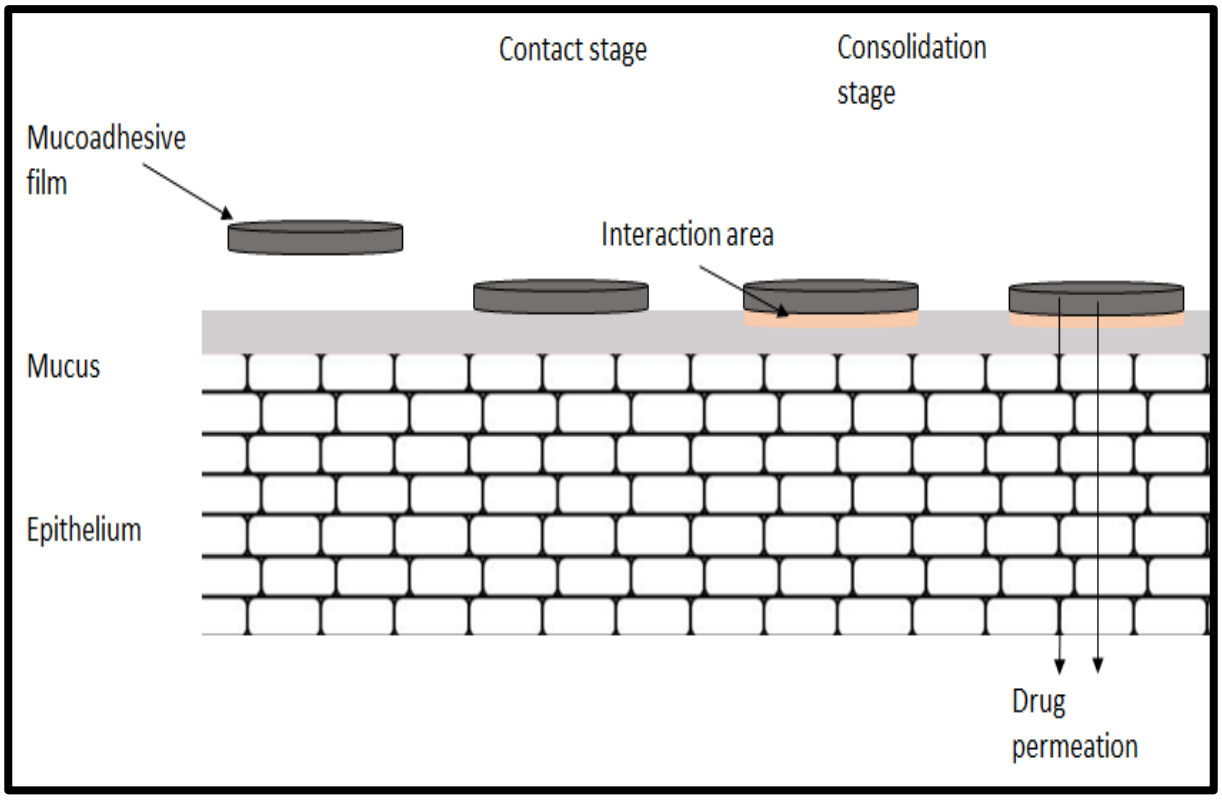


Figure 5. 1 Contact stage and drug permeation from buccal film

Solvent film casting is a very simple and cost-effective method, whereby the drug and excipients are dissolved into a solution, usually with a mixture of water and another suitable solvent for non-water-soluble excipients such as ethanol. This forms a viscous solution, allowing it to be spread across (casted) on a surface with more control. The solution is then left to dry into a film, after which can be collected and manufactured into its desired product formulation (Morales and McConville, 2011; Karki et al., 2016; Barnhart, 2008).

The HME is a process that requires much more sophisticated equipment, software and requires sufficient training for operation of the equipment. However, this method is a continuous process that provides better content uniformity and less unit operations are needed when compared to solvent film casting. The process involves mixing the drug and its carriers in their dry state as the first step. Once mixed, the mixture is inputted into the HME hopper and passed through its heating chambers to undergo melting. The melt is then extruded into a die, which will shape the melt into a film, after which it can be left to cool and dry for collection (Repka et al., 2007; Morales and McConville, 2011).

The effectiveness of the drug delivery profile of buccal formulations vastly depends on the mucoadhesive properties of the formulation. Mucoadhesion, in pharmaceutical terms for buccal administration, is the two-part mechanism that describes the attractive interaction between the components of the formulation and the mucous membrane of the buccal region. The first part is known as the Contact Stage – which is the time of intimate contact between the buccal mucous membrane and the mucoadhesive buccal formulation. The second stage is the Consolidation Stage – this involves the drug and its mucoadhesive carriers penetrating either into the oral mucosa membrane or into the crevices of tissue in the buccal area (Smart, 2004; Morales and McConville, 2011; Smart, 2005). This two-part description is essential, as the ability of the drug and its carriers to penetrate the mucus layer will determine the how effectively the drug particles will be transported into the bloodstream and ultimately decides the overall therapeutic effect (Guo, 1994; Morales and McConville, 2011). Therefore, it is of great importance that the mucoadhesive properties of the formulation is properly characterised and understood through testing.

Both methods exhibit advantages and limitations during formulation preparation. Electrospinning (ES) is an evolving and maturing engineering method with extensive recent interest in the pharmaceutical arena (Nazari et al., 2017; Mašek et al., 2017; Bhardwaj and Kundu, 2010; Mehta et al., 2017). This one-step technique has been developed to fabricate fibrous films loaded or co-loaded with API (at required quantities in-situ) at the ambient environment (Mehta et al., 2017).

Currently available rapid disintegrating buccal formulations such as tablets and patches have limitations relating to the short residence time at the absorption surface (Madhav et al., 2009; Al-Dhubiab et al., 2015). Here electrospinning films and cast films were proposed to assess their physical, chemical structure, and potential to release the molecule for prolonged period of proposed time (Salamat-Miller, Chittchang and Johnston, 2005; Al-Dhubiab et al., 2015; Dixit and Puthli, 2009). Therefore, the development of drug-loaded nanofibers may can overcome this problem by enhancing the surface area for interaction based on grooves and total surface exposure.

5.2 Aims and Objectives

The aim of this study was to investigate the effect of the technology on the film preparation. Electrospinning and cast method were carried out. Prepared films were analysed to determine their structure, morphology, thermal, and chemical properties of produced films.

Formulation system could influence the bioavailability, permeability and residential time of drug. Therefore, the aim of this chapter is to assess the films prepared by electrospinning and cast process.

The result fibre and cast films were analysed by employing thermal analysis using differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA); spectroscopic analysis employing Fourier transform infrared (FT-IR) and Raman spectroscopy. The morphology of fibres and cast were examined using Scanning Electron Microscopy (SEM). The sample preparation method was described in chapter 2.

5.3 Materials and methods

5.3.1 Materials

The information related to materials used in this chapter is in chapter 2 (method and materials)

Table 5. 1 Formulated fibres-sample compositions and their drug content.

Formulation		Polymer (5%w/v)	Drug (5%w/w)	Co-polymers (%w/w)		Drug Content (%w/w)
Electrospinning	F1	Polyvinylpyrrolidone (PVP)	indomethacin	-	-	4.98 ± 0.01
	F2	Polyvinylpyrrolidone (PVP)	indomethacin	Ethocel (E10) (5%)	-	4.8 ± 0.05
	F3	Polyvinylpyrrolidone (PVP)	indomethacin	Ethocel (E10) (5%)	Tween 80	3.4 ± 0.21
Cast	F4	Polyvinylpyrrolidone (PVP)	indomethacin	-	-	4.98 ± 0.02
	F5	Polyvinylpyrrolidone (PVP)	indomethacin	Ethocel (E10) (5%)	-	4.94 ± 0.03
	F6	Polyvinylpyrrolidone (PVP)	indomethacin	Ethocel (E10) (5%)	Tween 80	4.92 ± 0.02

5.3.2 Methods

Fibres and cast preparation methods is as same as the method mentioned in chapter 2 (methods and materials). All preparations contained PVP (5% w/v) and INDO (5% w/w of PVP). Other excipients were added in quantities shown in Table 5. 1 as a function of w/w % of PVP.

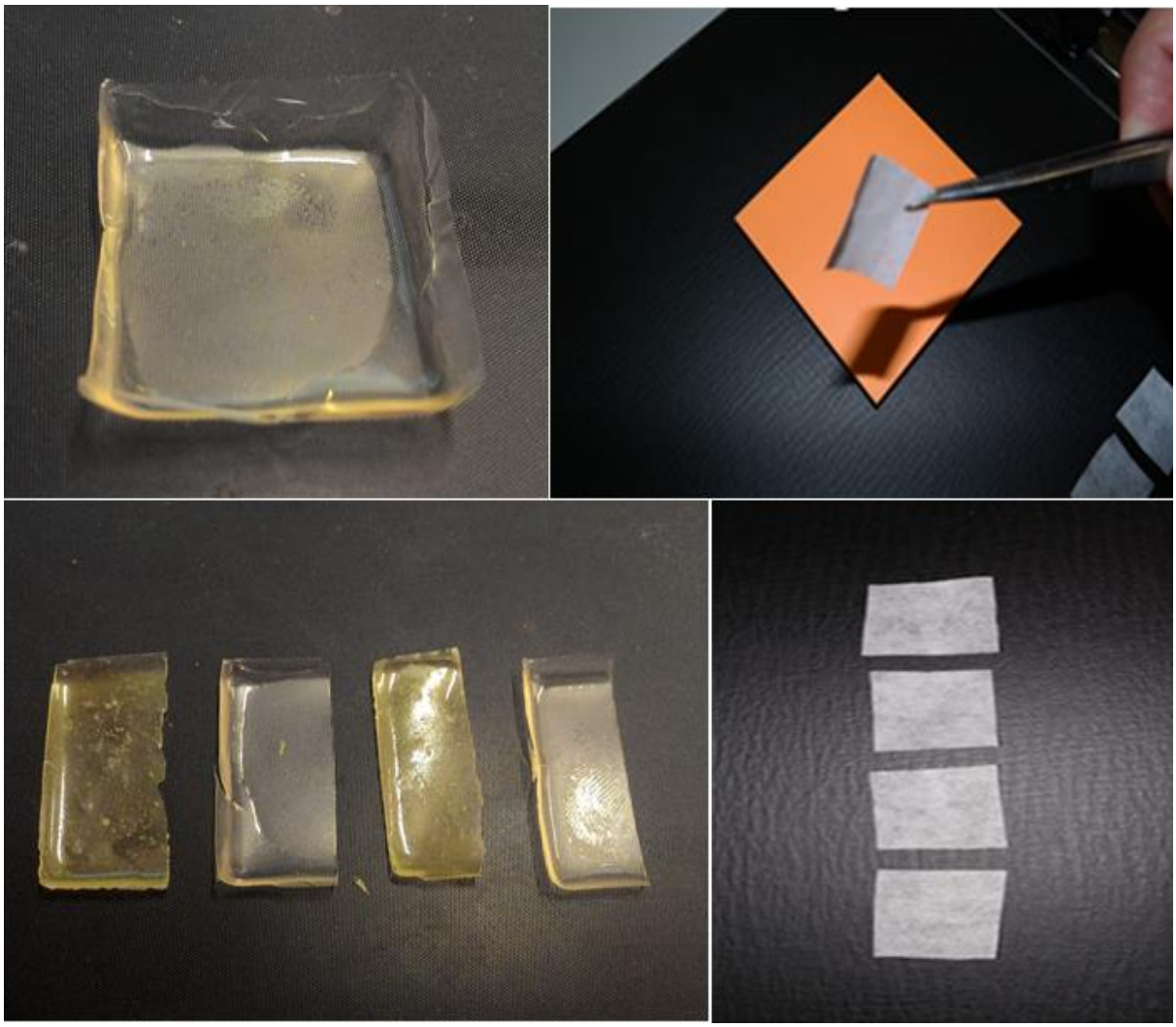


Figure 5. 2 Digital image of prepared cast (left) and fibre (right)

5.4 Results and discussions

Figure 5. 3 SEM images of formulated fibres taken at X25K and cast image X5K magnification.shows digital image of surface morphology of formulated electrospun and cast films. All fibres exhibit smooth surface morphology with evident variation in fibre size and quality distribution between samples. F1 shows more bubble like in the fibre compare to others (F2 and F3). These arise due to formulation composition (e.g. co-polymers). Furthermore, the mean fibre diameter was found to increase with adding co-polymers to the formulation. Fibers produced using Ethocel (E10) 5 w/w % showed slightly increase in diameter, although mostly less than 300nm. On the other hand, formulated fiber with added second co-polymer (Tween 80) presented mostly in range 300-600nm (Figure 5. 4).

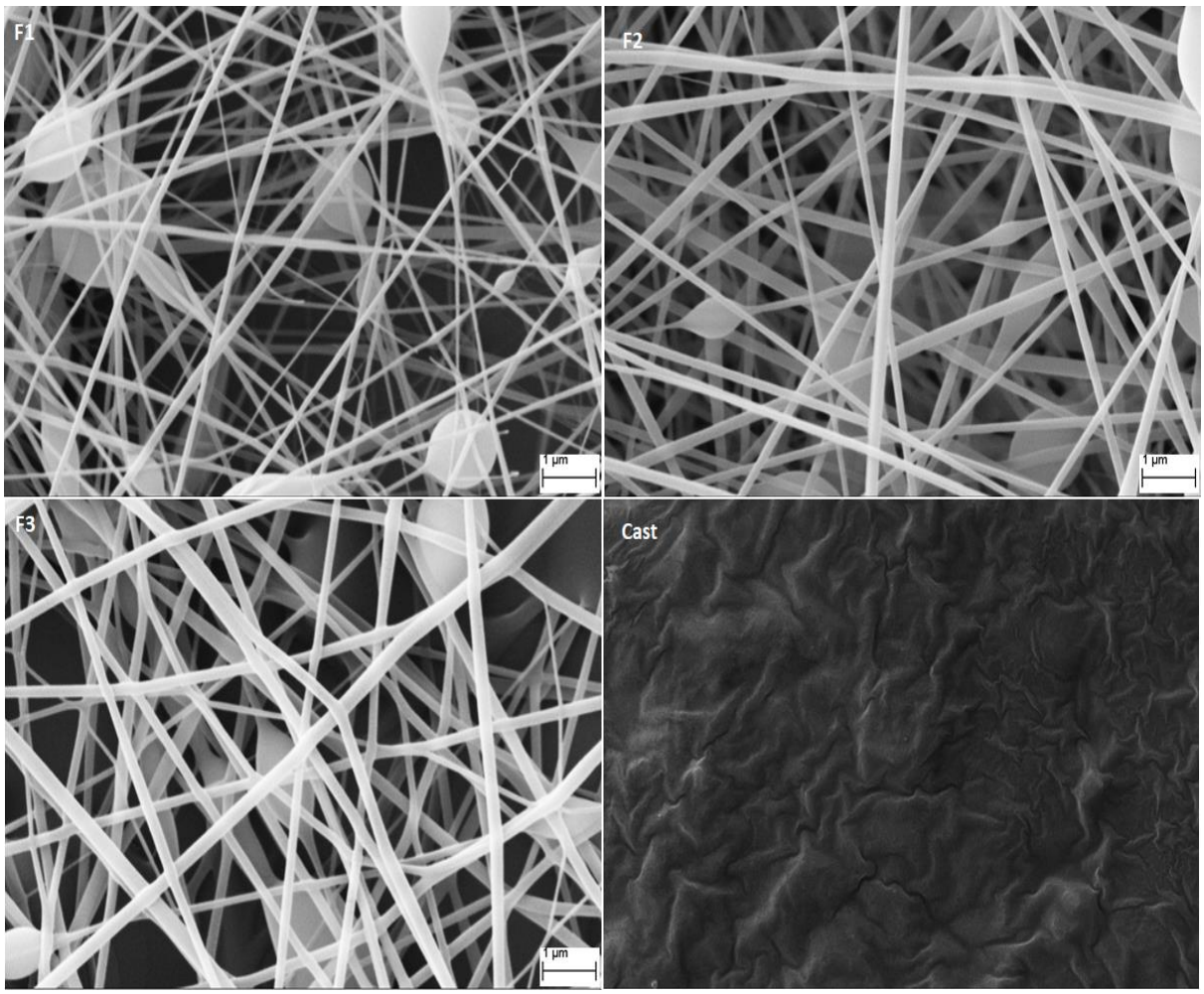


Figure 5. 3 SEM images of formulated fibres taken at X25K and cast image X5K magnification.

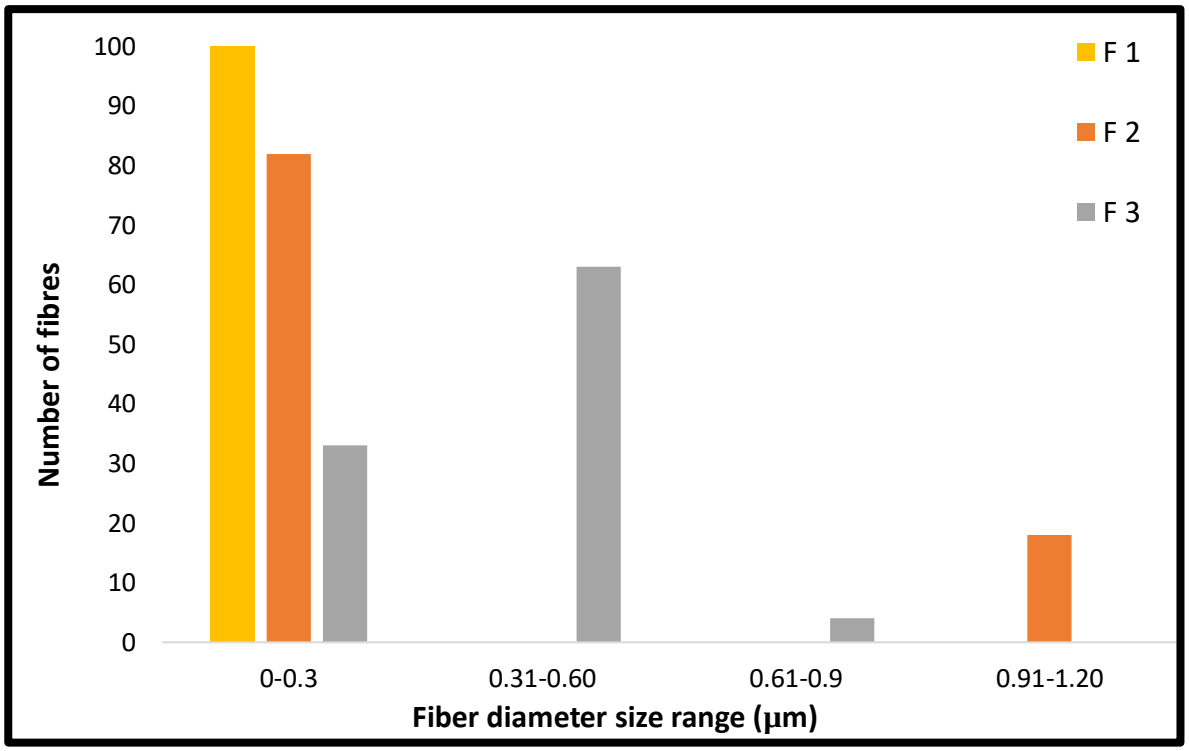


Figure 5. 4 Diameter size distribution histogram of 100 fibers of F1, F2, and F3.

DSC is one of the thermal analytical technique which allows for direct study of thermal stability of drugs. Heat flow between a sample and an inert reference sample during a specified temperature is monitored. DCS thermograms are shown in Figure 5. 5 (A and B). INDO is a crystalline drug with a melting temperature ($\mathbf{T_m}$) of 163.5 °C which is shown at ~ 164 °C in chapter 3.

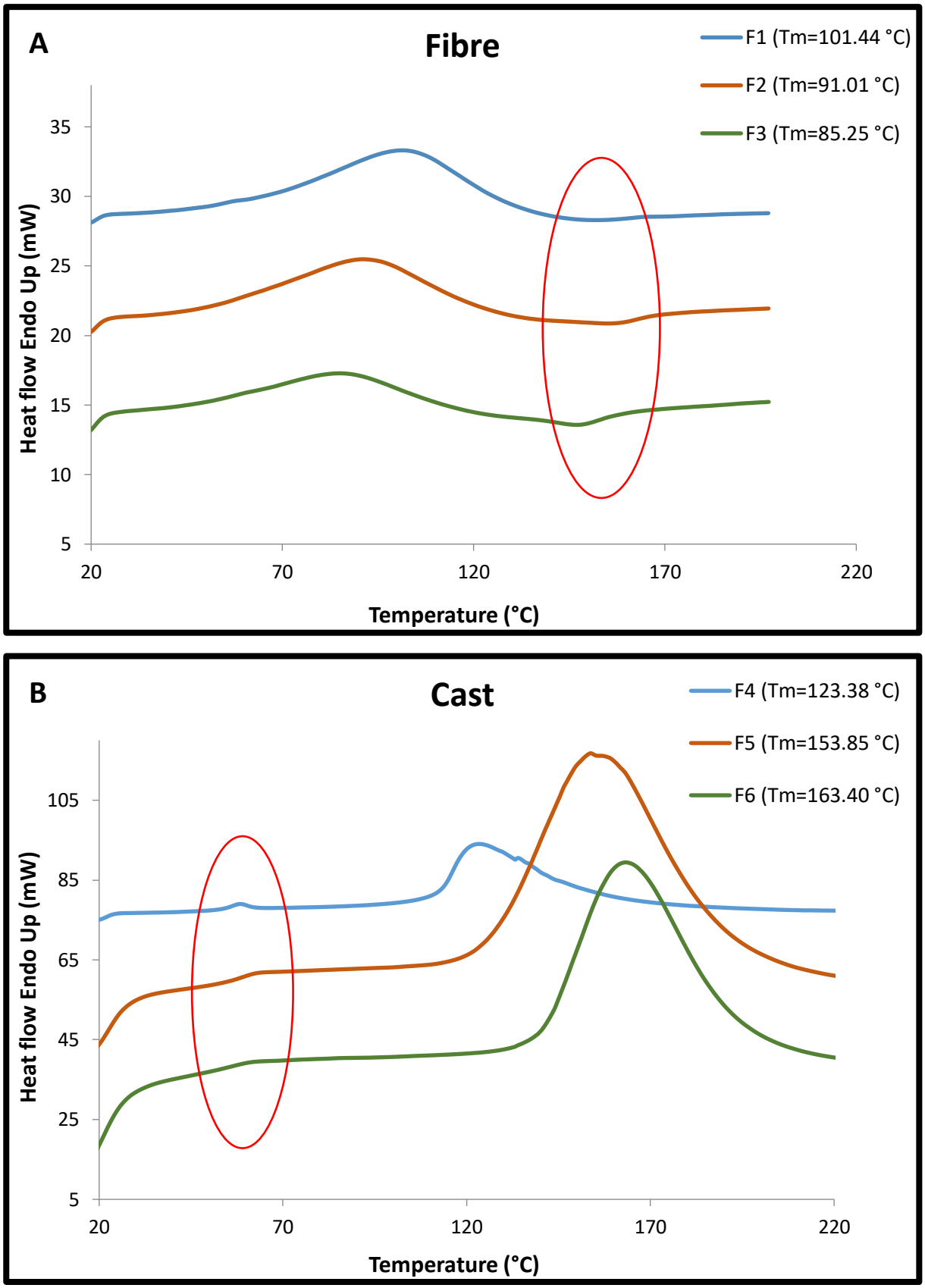


Figure 5. 5 DSC thermogram of A) formulated electrospun films and B) formulated cast films.

The melting temperature of formulated INDO/PVP electrospun films decreased significantly once compared to raw INDO, raw PVP and physical mixture of INDO/PVP (chapter 1). This difference corresponds to INDO being molecularly dispersed within the fibrous polymeric films increasing the amorphous state of the active. The melting point of electrospun films was higher for INDO/PVP polymer and reduced with increasing co-polymer content which might indicate increased amounts of polymer in the formulation providing a greater polymeric matrix volume for INDO dispersion and spacing.

A clear alteration is also noted between electrospun fibrous films Figure 5. 5 (A and B) and cast films and the corresponding raw materials in the formulations. Since the cast do not enable INDO dispersion as efficiently as electrospun films, their melting temperatures are closer to the T_m values of neat drug. The combination of INDO, PVP and Ethocel (E10) 5% (F2) and INDO, PVP, Ethocel (E10) 5% and Tween® 80 (F3) showed an exothermic peak around 160 °C and 145.32 °C respectively, which is credited to residual solvent entrapped within fibres. Though the insensitivity of exothermic is slightly higher for F3 formulation.

TGA is another thermos analysis technique which provides very useful and relevant information for materials of interest by measuring and recording melting point, transition temperature and weight loss of materials. TGA was employed to observe the thermal degradation of electrospun and cast films. The previous studies (chapter 1-4) have shown both neat electrospun samples and their drug loaded (INDO) counterparts to exhibit similar degradation steps.

All formulations (electrospun) show two degradation steps; the first is weight loss observed at temperatures below 100 °C, which is attributed to the evaporation of the residual solvent or adsorbed water in fibrous films. The second major weight loss is observed from 350 to 500 °C, which is ascribed to the decomposition of PVP and other co-polymer excipients. However, electrospun formulations with co-polymer (F2 and F3) show earlier degradation compare to F1.

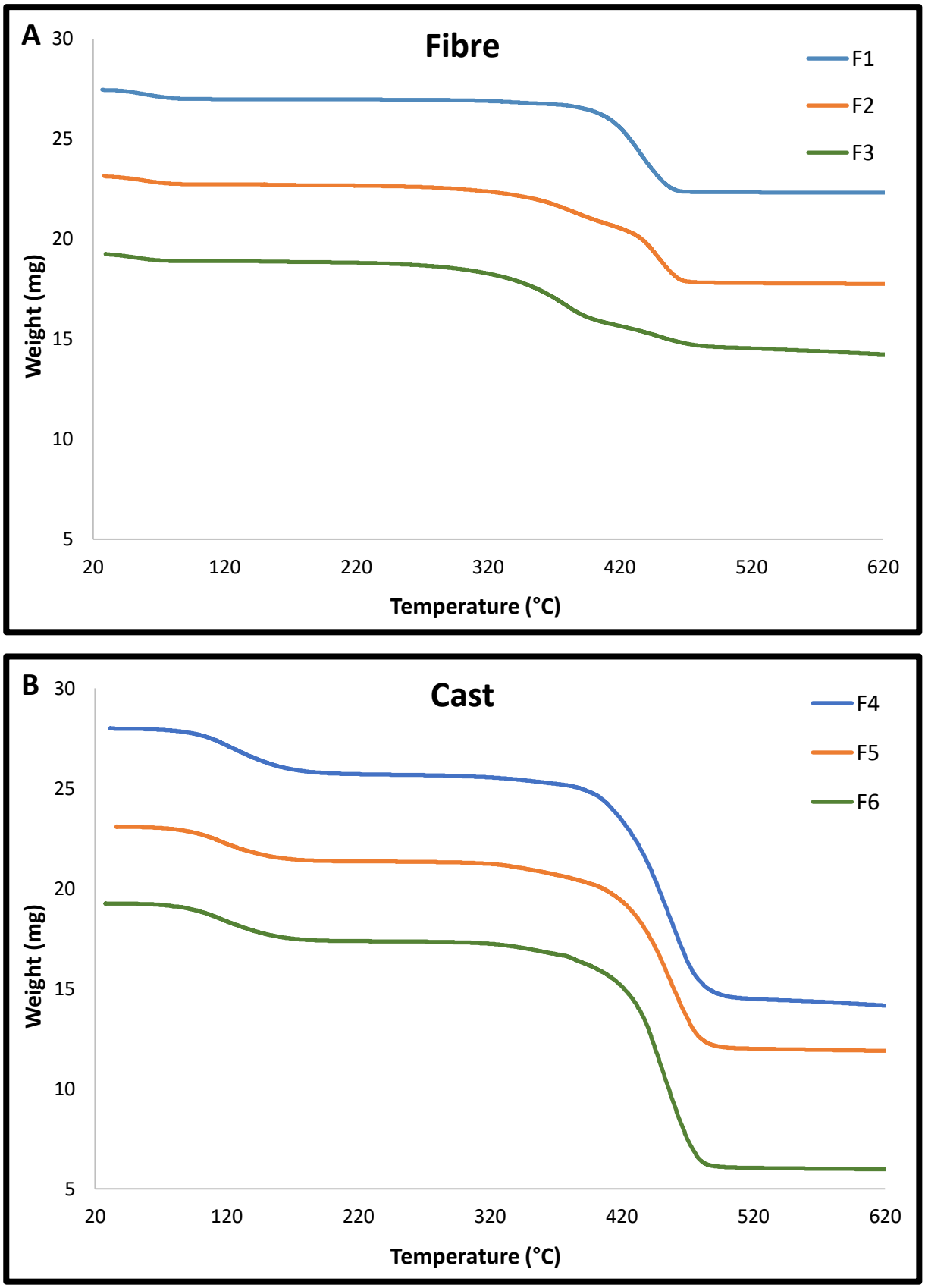


Figure 5. 6 TGA curves of A) formulated electrospun films and B) formulated cast films.

TGA thermograms also confirm INDO loading into filamentous matrices. As has been reported in a previous study, a shift in the maximum temperature associated with weight loss can be used to demonstrate drug entrapment within a fibrous system. This is indicating thermal degradation can be expedited or delayed based on the selected active or co-polymers embedded within the fibrous film. As shown in Figure 5. 6, the maximum thermal degradation values of the various drug loaded systems are different from neat INDO and PVP (chapter 3).

Figure 5. 7 (A and B) shows FTIR spectra for F1, F2, and F3 electrospun samples and F4, F5, and F6 Cast samples with different co-polymer concentrations in the range 4000–400 cm^{-1} respectfully. The FTIR spectra of samples comprising INDO, PVP and other polymers show various characteristic peaks. These are attributed to vibration modes of the aromatic ring in INDO (particularly amide **ν**C═O at 1600 cm^{-1}), various peaks of PVP particularly the **ν**C═O at 1680 cm^{-1} which shifted to 1654 cm^{-1} as a result of hydrogen bond formation (Borodko et al., 2006). Other absorption peaks are recorded at 3490 (O—H), 2983 (C—H), 1920 (C═C) and 1425 cm^{-1} (C═C).

The changes observed in the INDO, PVP and co-polymer sample spectrum exhibit near identical peaks as INDO and PVP with a slight change to peak intensity at 1654 cm^{-1}. This arises due to Van der Waals force of attraction or hydrogen bonding between co-polymers. New absorption bands were detected in formulations containing co-polymers; apparent at 1087–1113 cm^{-1} and these are attributed to carboxylic acid carbonyl stretching in co-polymers.

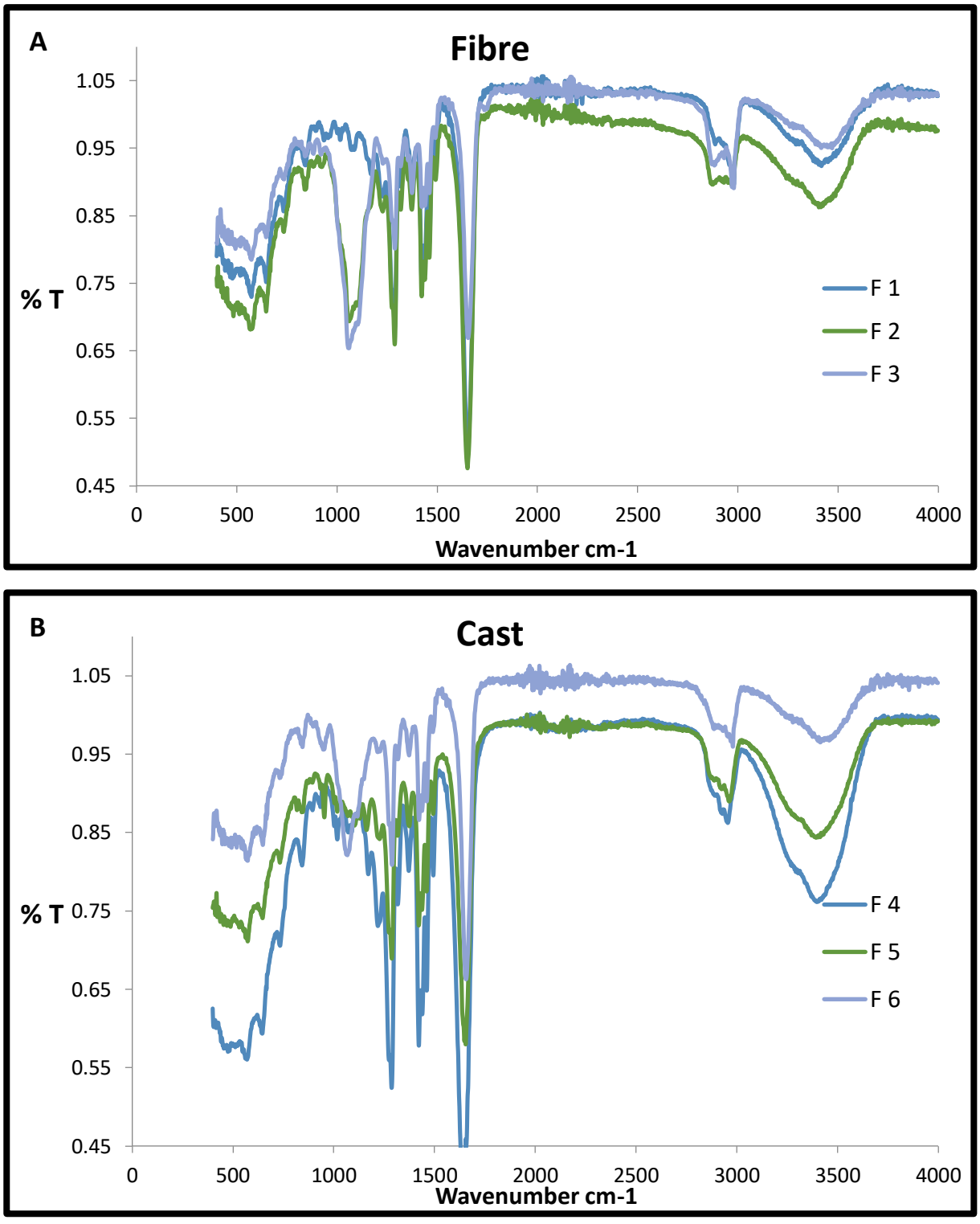
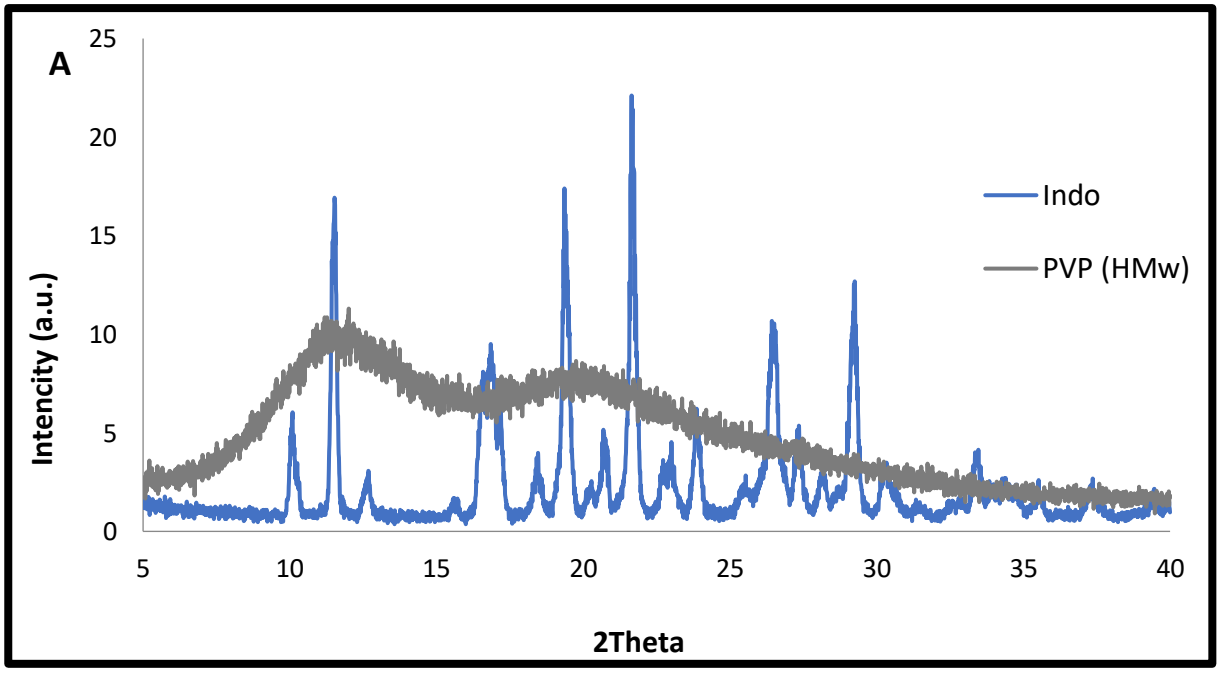


Figure 5. 7 FTIR spectra of formulated A) fibre films and B) casts.

XRD patterns of formulated electrospun films and cast films are displayed in Figure 5. 8. The diffraction spectrum of pure INDO powder (Figure 5. 8A) demonstrates that the drug is highly crystalline and possesses multiple diffraction peaks at (~ 2 theta = 10–38°) whereas, PVP shows a broad amorphous band. All principle peaks of INDO (11.7, 16.7, 19.5, 21.6 and 21.6) disappeared in the diffractogram of electrospun INDO with PVP and various other **co**-polymers. This indicates INDO is dispersed throughout the fibrous matrix and is in the amorphous state. On the other hand, all cast formulations show peaks at 29, 32, and 38 (Figure 5. 8C). This indicate the crystallinity of cast prepared formulations.

The amorphous state provides several benefits and limitations for drug dosage forms. Integrating active in the amorphous state has shown to expedite drug release and bioavailability through enhanced solubility. However, this limits sustained release features and could also inadvertently result in crystallization and other stability issues.



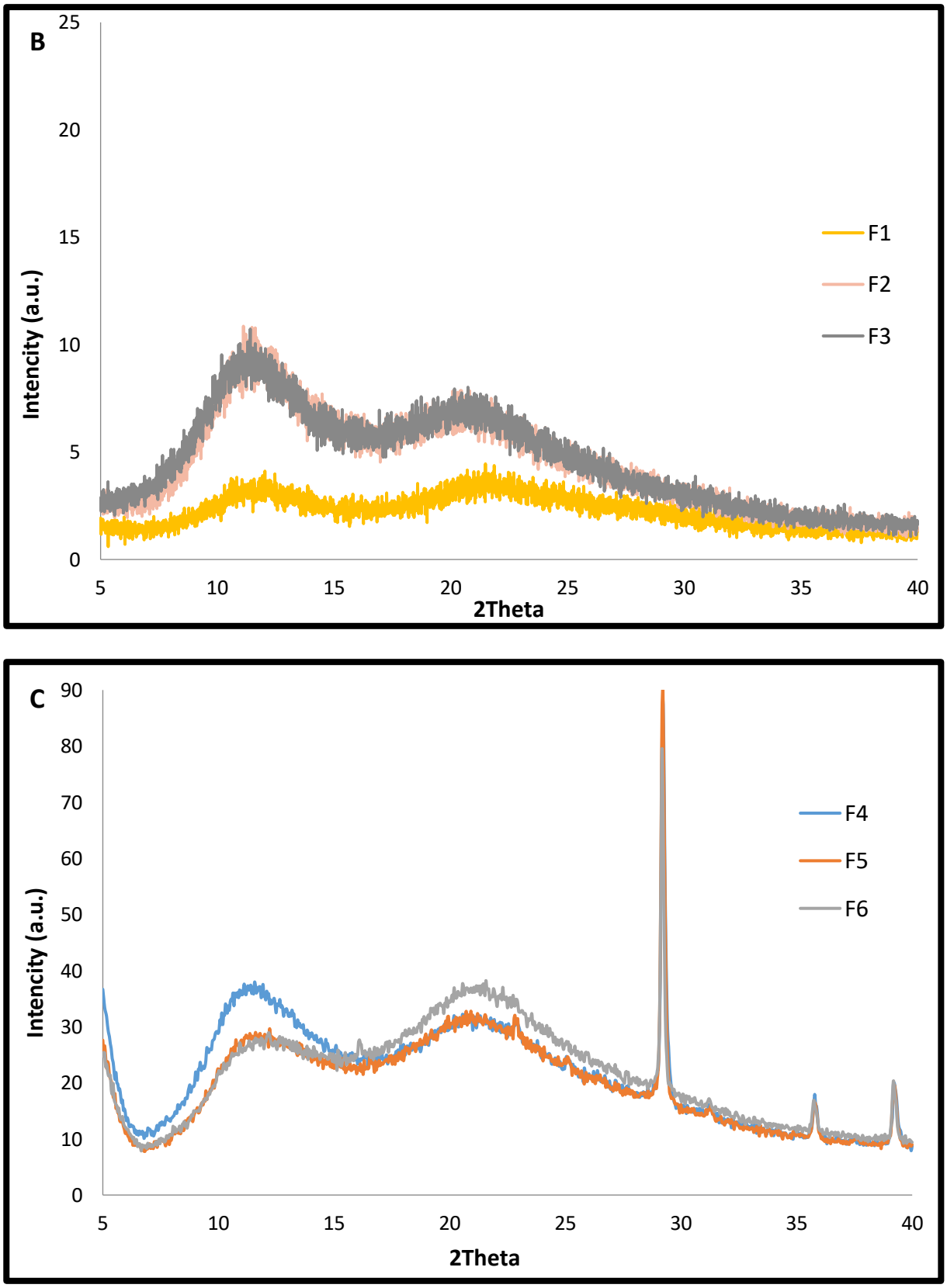
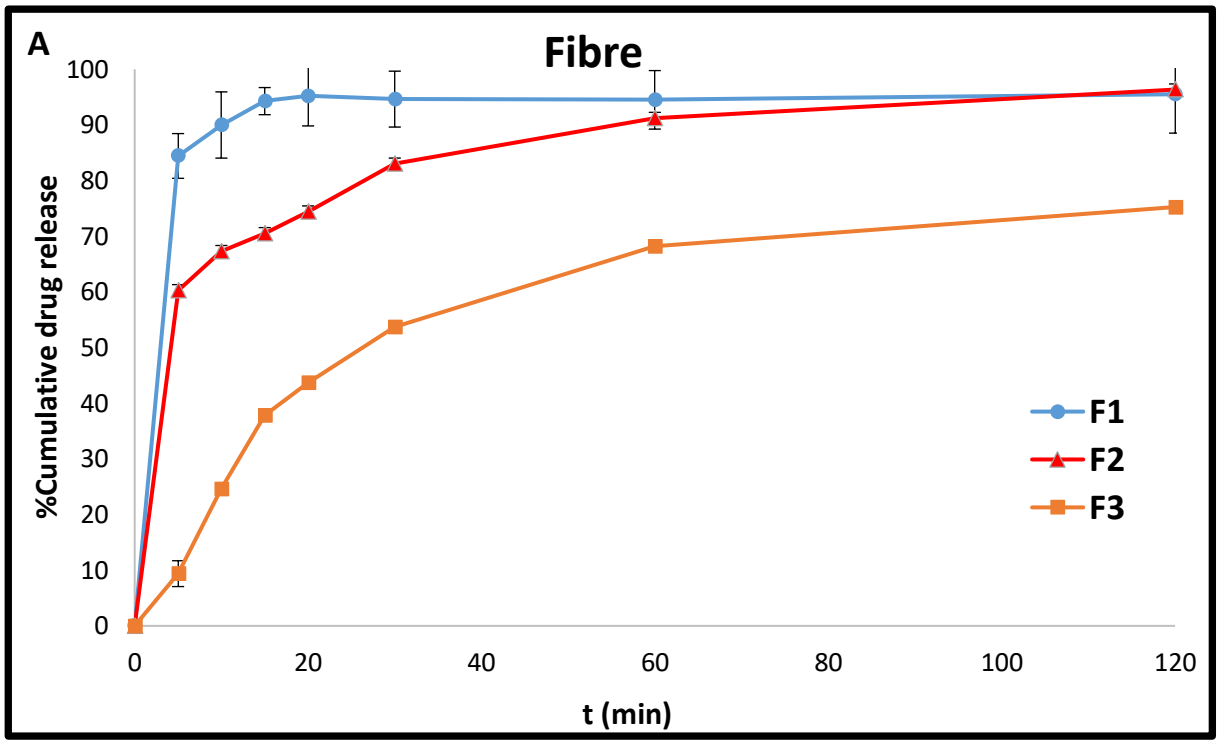


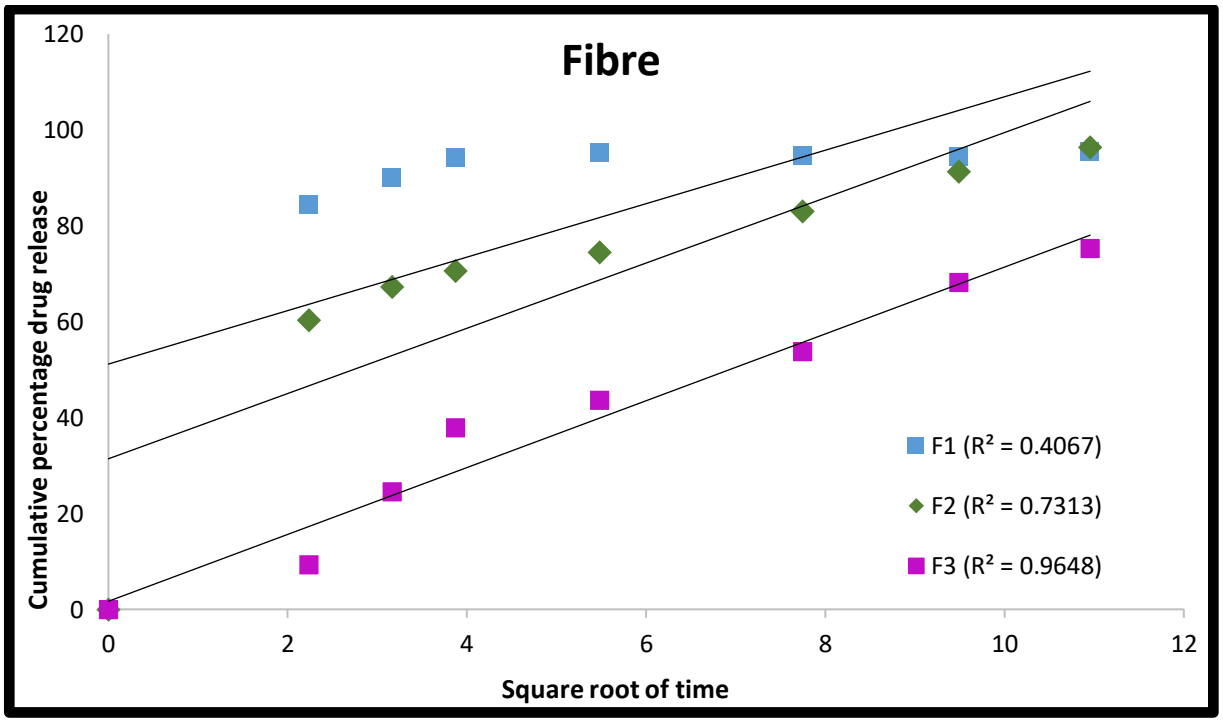
Figure 5. 8 X-ray diffractograms of A) INDO and PVP, B) formulated electrospun films, and C) formulated casts.

The drug content in various film dosages ranged between 3.4 and 4.9% w/w which is less than the theoretical maximal value (5% w/w). This may be attributed to drug precipitation. Figure 5. 9 illustrate the cumulative release profiles for all formulated fibrous samples and cast samples respectively. A reduction in the release rate of INDO was observed by adding water insoluble polymer (Ethocel (E10)) in the formulated systems (Figure 5. 9). The effect of ethyl-cellulose content on drug release from polymer beads containing INDO has been shown, where polymer cause reduction in the release rate of the active (Suryakusuma and Jun, 1984) (Suryakusuma and Jun 1984). This is explained due to the non-soluble nature of the material which limits water interaction with the polymeric matrix. The presence of Tween® 80 in samples reduced the release rate further when compared to its congener prepared with Ethocel (E10) 5% w/w. A steep and fast release was recorded for all formulations within the first 5 minutes followed by a steady increase. Formulations (F1 and F2) showed faster release compare to F3 and reached up to 90% of their release. However, F3 release reached up to 70%.

On the other hand, cast formulations recorded slower release compare to same formulation in electrospun form. The greatest release of INDO from casts (~ 90%) was observed for formulations F4 and F5. F6 recorded about 70% of release. The variation release profile of formulation is due to attribution of higher viscosity of Ethocel (E10) compared to the formulations with no co-polymer and therefore, resulting in prolonged release of the active over time.

The kinetic release model of PVP/INDO/co-polymer nanofibers and cast are shown in Figure 5. 9 where the cumulative quantity of drug released per square centimeter of fibrous films is plotted against time and fitted to the Higuchi model. It was observed that the release profile of INDO from fibrous sample F3(R^2 = 0.9648), F4 (R^2 = 0.9522), F5 (R^2 = 0.9515), and F6 (R^2 = 0.939), suggest that the release of the drug in these formulations were governed by diffusive mechanisms. This implies INDO release is based on Fickian diffusion, based on drug permeation through the fibrous polymeric network (Neo et al., 2013).


A
Fibre
%Cumulative drug release
t (min)
F1
F2
F3


Fibre
Cumulative percentage drug release
Square root of time
F1 (R² = 0.4067)
F2 (R² = 0.7313)
F3 (R² = 0.9648)

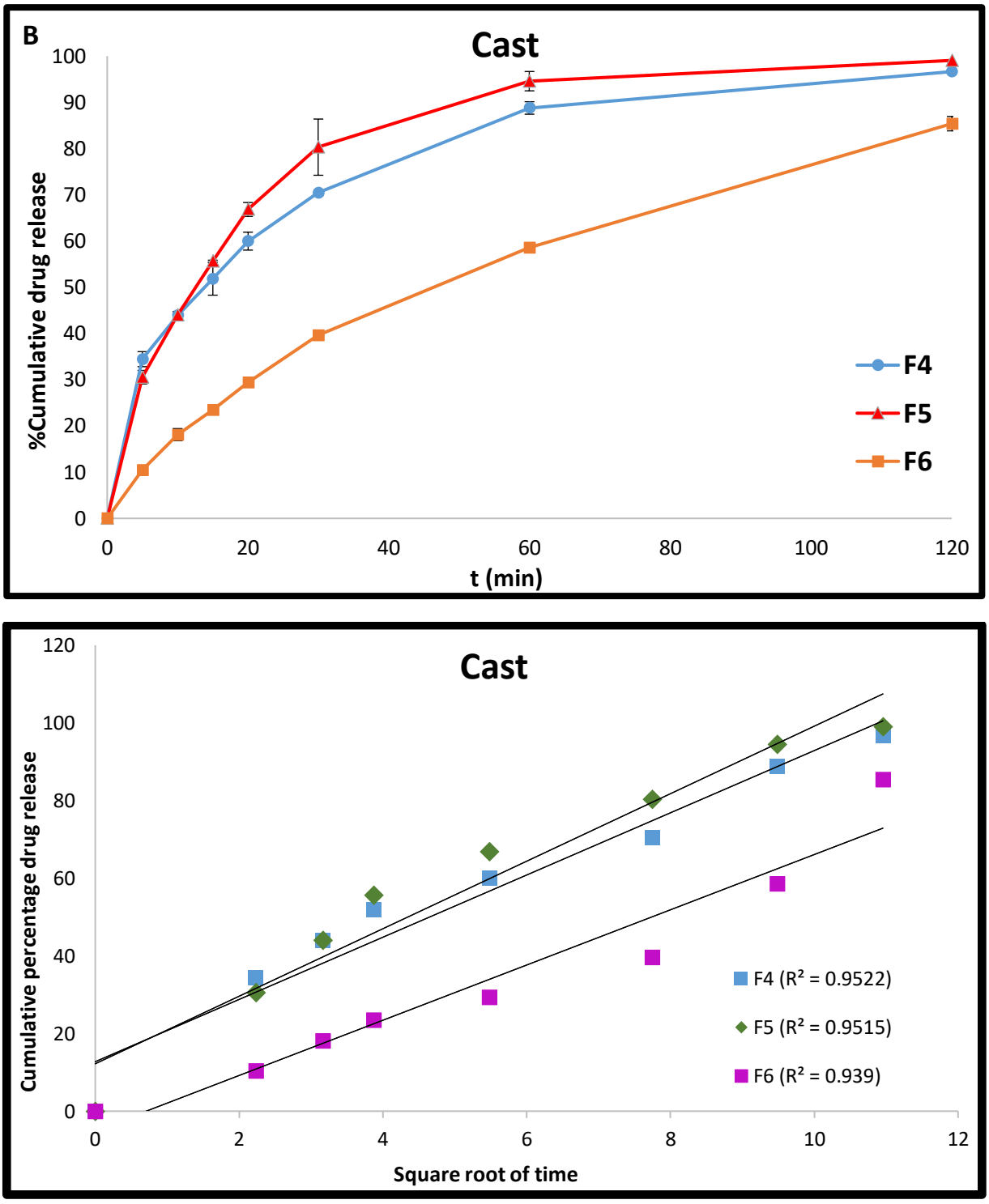


Figure 5. 9 Release profiles of A) F1, F2, F3, and B) F4, F5, F6 formulations in buffer pH 6.8. A1 and B1 show plots of cumulative release against root time for corresponding formulation.

5.5 Conclusion

The buccal route of administration is a specific type of drug delivery that involves the patient placing the drug in the gap between the gum and the cheek until it completely dissolves. It is a promising administration route since it avoids destruction from the gastro-intestinal tract and first-pass metabolism by the liver, thereby providing better bioavailability with less wastage and possible harmful intermediates being produced from these reactions. It as an alternative to other delivery system such as nasal or skin. Penetration of active pharmaceutical ingredient (API) is with ease due to physiology of the buccal mucosa. Moreover, it has rapid cellular turnover and recovery. Also, film casting, and hot melt extrusion has been successfully explored for mucoadhesive buccal films but still many possibilities stay in the design of buccal films. Currently under research however, is the formulation of buccal films by making drug-loaded fibres via electrospinning. However, preceding aim of pharmaceutical scientists with new improving technology is to manufacture patient safe and friendly dosage form.

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Chapter 6 Quality by design of Nano-pharmaceutical fibres

6.1 Introduction

Quality by Design (QbD) is a statistical analytical tool that uses sound science and risk-based knowledge to approach experiments and projects with the goal of achieving a product that can be consistently manufactured to its desired level of safety, efficacy and quality. Therefore, adequate control is required to assure the manufacturing process is performed to a high level of accuracy, robustness and reliability. In recent years, the pharmaceutical industry has highlighted, risk management and quality by design (QbD) as the importance of product quality has elevated. With application of nanotechnology and nanomaterials to drugs, massive improvement in health challenges such as bioavailability, biodistribution, drug action, degradation and targeted delivery area has been achieved. However, there will be risks associated with formulation and the materials used and therefore, the need for an approach to address them is necessary. QbD prevents alteration of quality and safety of new formulation or new drug by identifying, analysing and control all parameters that might affect the process (Bastogne, 2017; Pramod et al., 2016; Colombo et al., 2018).

Important QbD objectives in pharmaceutical are as: a) consistent production of high quality products by design the processes and manufacturing, b) identification and control process parameters and important component to determine the critical quality attributes (CQAs), c) reduce the deviation to improve process capability and d) to tackle the cause of problems and manage changes after approval. (Yu et al., 2014; Yerlikaya et al., 2013; Ko et al., 2018; Pramod et al., 2016). Process design is the key focus of the QbD. Risk based approach and the relation with QbD has been discussed in ICH Q8, Q9, and Q10 (Ko et al., 2018; Thakur, Kaur and Sharma, 2017; Hubert et al., 2014a; International Conference on Harmonization (ICH), 2009a; International Conference on Harmonization (ICH), 2009b; International Conference on Harmonization (ICH), 2008)

Quality by Design (QbD) is a risk-based tool used by many pharmaceutical industries with the goal of ensuring the quality of the final product by improving product and process understanding (Sangshetti et al., 2017a). This is because a better understanding will enable better process control to be achieved (Rathore and Winkle, 2009; Bastogne, 2017). When good process control has been established, the manufacturing process will become more robust, efficient and cost effective, all while reducing the variability in quality during all manufacturing

stages, which will ultimately guarantee the end product quality (Yu et al., 2014; Ko et al., 2018; Wu and Khan, 2009; Stults et al., 2015; Rahman, Siddiqui and Khan, 2013).

The foundations (green), stages (red chevrons) and enablers (yellow) of a QbD approach are summarised in Figure 6. 1. It emphasises the importance of using prior knowledge (knowledge already known about the process/product) alongside the new understanding of the process and product through a risk-based approach to form the foundations (Vogt and Kord, 2011; Rozet et al., 2013).

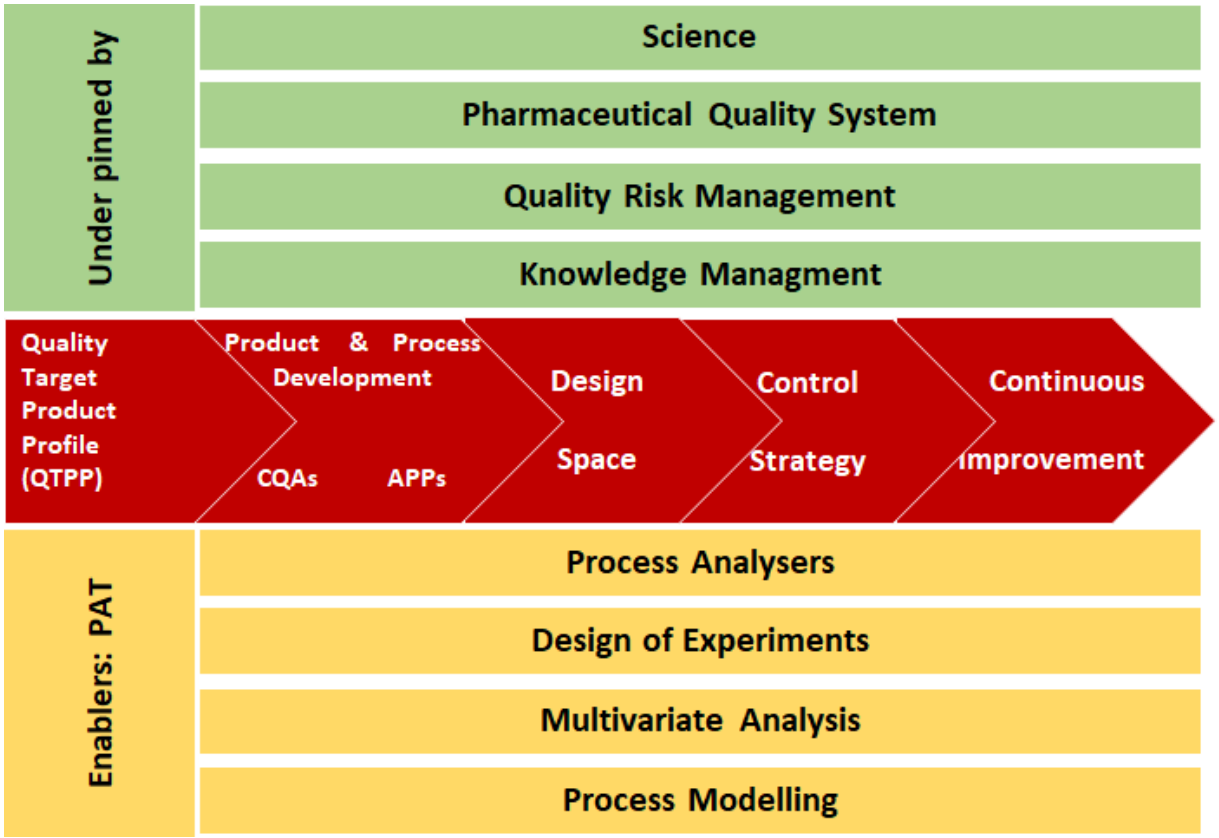


Figure 6. 1 Roadmap and stages of QbD

The use of Process Analytical Technique (PAT) tools helps to further enhance QbD elements in experiments. PAT tools are convenient instruments that can be used to take measurements, whether in-line, at-line, on-line or off-line (Sangshetti et al., 2017b). Statistical software programmes, such as JMP, can also be used as a PAT tool, which is capable of performing multivariate analysis, which involves assessing how multiple parameters can affect the responses of interest (the variables or characteristics that can greatly affect the product quality) simultaneously. PAT can aid in gaining information that may be applied towards modelling of the process, further improving process understanding. A Design of Experiment (DoE) can be

constructed, which is a pre-planned experimental design that aims to describe the relationships of the input variables, process parameters and the responses (output variables). The number of runs required to perform the experiment depends on what design is used to make the DoE, how many independent factors are being assessed and the number of centre points included in the design. Centre points allow curvature to be recorded in the results, which helps to further understand the relationship between the factors and responses (Dispas et al., 2018; Hubert et al., 2014b).

The stages of the QbD process are outlined in the chevrons in Figure 6. 1. The first stage is to create a Quality Target Product Profile (QTPP), which is essential for a QbD approach as stated in the ICH Q8 (International Conference on Harmonization (ICH), 2009a). The QTPP defines the requirements of the end product characteristics and which of these are critical to manufacturing the product to its required efficacy, safety and quality so that the patient may be satisfied with the product. Therefore, the QTPP serves as a collection of properties and their acceptable ranges/values of a model product for the pharmaceutical chemists to target (Sangshetti et al., 2017a; Zhang and Mao, 2017).

The Critical Quality Attributes (CQAs) are the physical, chemical, biological and microbiological properties of the product and its intermediates that are considered to have potential to greatly affect the quality of the final product (e.g. electro conductivity, surface tension). Therefore, it is of paramount importance to consistently monitor and control these CQAs. Critical Material Attributes (CMAs) also link in with CQAs and are defined as the physical, chemical, biological and microbiological properties of the input materials used in the experiment (e.g. polymer molecular weight). These CQAs can be identified by studying the QTPP in conjunction with performing risk assessments. Risk assessments can be done by providing risk scores through a traffic light system or Ishikawa diagram (Figure 6. 3).

Critical Process Parameters (CPPs) are the parameters of the process (e.g. flow rate, voltage) that are critical to the final product quality. As with CQAs, they can be identified through risk assessments, such as a traffic light system of CQAs against CPPs. They also need to be well controlled and monitored throughout the process, as this helps to control the CQAs too. PAT tools can aid in the control of parameters so that the CQAs can be maintained within their critical limits. This can work by the tool alerting the operator that a particular parameter or CQA is outside of its limits, enabling the operator to manually change the parameter to its appropriate settings (Zhang and Mao, 2017). Another way is that the PAT tool could be set up

so that it can monitor the parameters and relevant CQAs and automatically feedback or feedforward the information, via proportional, integral and derivative (PID) control, to allow the system to 'fix itself' if a parameter or CQA is outside of its pre-determined limits.

The design space is defined as the multidimensional space (space created by the relationships between multiple variables), where working within this space is not considered a change and the parameters and CQAs are within limits to ensure its desired quality levels are met. Although a design space is optional, its benefits are certainly invaluable and offers a range of choices that can be chosen to suit the operator's need. A design space cannot be created until results have been obtained. This is because sufficient knowledge is required to create the design space, as shown in Figure 6. 2, where the design space is within the knowledge space. If part of the design space is outside the knowledge space, then there will be some grey areas where there is poor understanding about a particular aspect of the process or product, and so further testing would be required to gain more knowledge about this. The normal operating space is the space in which the operator has decided to work within, while still inside the design space. This particular space may be chosen to further guarantee the quality of the product or it may be that these parameter limits offer more efficient and cost-effective settings whilst maintaining the end product quality (Sangshetti et al., 2017a). The design space can be demonstrated in different forms, also a 3D response surface plot can give more information about the relationship between the variables, while a 2D contour plot can give straightforward detail on the variable settings that will be within the design space.

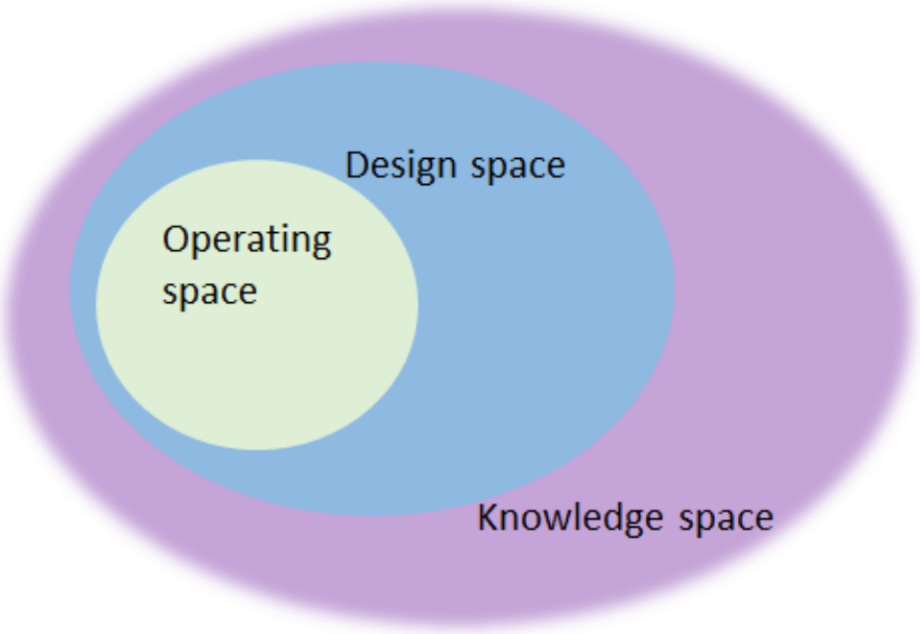


Figure 6. 2 Different spaces associated with the design space

With all of the above information obtained, a control strategy can then be devised. This is a planned set of controls taken from current process and product understanding to further ensure the process performance and product quality is of appropriate standards. The control elements involve, but not limited to, input controls, process controls, and testing controls of unit operations and CQAs of the product. This is an essential part of QbD that helps to summarise the controls that must be established during the manufacturing process (Sangshetti et al., 2017b).

Continuous improvement is the final stage, which can go on for as long as is necessary. This is because the process can be improved in future to promote cost-effectiveness, efficiency, robustness, time saving changes and product quality by periodically reviewing and assessing the process and product understanding, the. Also, new technological advancements will be developed that may be applicable to the manufacturing process of interest (Zhang and Mao, 2017).

Nevertheless, a very small number of studies have been conducted where Quality by Design (QbD) have been incorporated in the experiments of researches involving electrospinning. Therefore, in order to obtain better process and product understanding, QbD is required to be included in electrospinning projects.

6.2 Aims and objectives

The aim of this project is to apply the concepts of QbD to the electrospinning of NSAID drugs and determine and control the most significant factors that can affect the quality of the fibre. Creating a design space and control strategy for the process is also a target to be achieved.

The objectives of this chapter are:

- To construct a Quality Target Product Profile (QTPP)
- Perform Risk Assessments to identify Critical Quality Attributes (CQAs) and Critical Process Parameters (CPPs)
- Construct a Design of Experiment (DoE), where the factors to be assessed that can affect the product quality are: Flow rate, voltage, distance between electrospinning needle and collector and drug used in formulation; and the responses to monitor are: fibre diameter, fibre quality and drug release profiles
- Perform the experiments and obtain results
- Analyse results and use statistical analytical software to develop a design space and create a control strategy

6.3 materials and methods

6.3.1 Materials

The materials that will be used in the proposed experiment are shown in Table 6. 1.

Table 6. 1 Formulated fibres-sample compositions and their drug content.

Formulation	Polymer	Drug	Drug Content (w/w %)
F1	Polyvinylpyrrolidone (PVP)	Diclofenac Sodium	4.68 ± 0.08
F2	Polyvinylpyrrolidone (PVP)	indomethacin	4.98 ± 0.01

6.3.2 Methods

Fibres preparation method is as same as the method mentioned in chapter 2 (methods and materials). All preparations contained PVP (5% w/v) and NSAID drugs (Indomethacin and Diclofenac Sodium) (5% w/w of PVP).

A full factorial Design of Experiment (DoE) was made with four factors at 2 levels each, two responses and four center points. Therefore, a total of 20 runs were performed, where each run was done for 1 hour. The details of the DoE dialog are shown in Table 6. 2.

Table 6. 2 Factorial Design of Experiment (DoE) dialog

DoE Component	Response or Factor	Lower limit	Upper limit	Centre Point dimensions
Fibre Diameter / nm	Response	None	<1000nm	N/A
Quality of Fibre	Response	1	5	N/A
Drug (Indomethacin and Diclofenac Sodium)	Factor	5% of formulation	5% of formulation	Two centre points for each drug
Voltage / kV	Factor	10	20	15
Flow Rate / µl/min	Factor	5	35	20
Distance between needle and collector / cm	Factor	5	20	12.5

6.4 Applying QbD

Prior to the proposed methodology, the initial applications of QbD must first be made.

6.4.1 The QTPP-intent design

Since a complete product will not be manufactured, the design intent of a QTPP was made, as shown in Table 6. 3. Here, a description of the quality attributes of the materials and product is given, along with their intended target limits and purpose and the level of criticality on how greatly they may affect the final product quality. From this, the primary CQAs that will be monitored are: fibre diameter, fibre quality and release profile.

Table 6. 3 Design intent of QTPP describing the criticality levels of the attributes that may affect the quality of end product.

Quality attribute	Target	Criticality
Product Form	Fiber	Critical
Fibre Diameter	≤ 2µm to maximise surface area	Critical
Release Profile	Slow or fast release (depend on intended use)	Critical
Fibre Quality	Structure and shape must be consistent throughout fiber	Critical
Impurities	Must be low as possible to avoid harm/instability	Not Critical
Pharmacokinetics	Must be appropriate	Not Critical
Solubility	Should be reasonable	Not Critical
Microbiology	Must be low and within limits	Not Critical
Stability	Must have consistent stability profile	Critical
Porosity	Must have high porosity to maximize surface area	Critical

6.5 Risk assessments

The initial risk assessment (RA) of the CQAs against the formulation variables of the process before applying controlled factors are fibres quality, fibres diameter, and release study. It becomes clear that most of the variables pose high risks towards the CQAs. Therefore, some of the variables require to be controlled to avoid results being impacted.

During the experiment all parameter kept constant and thereafter, the risk factors such as fibres quality, fibres diameter, and release study which are the main risk consideration of the CQAs against the formulation variables need to be evaluated.

It is evident that this greatly lowers the overall risk as the drug used will be part of the experiment and so will be deliberately changed to see the effect this may have on the fibres to be produced.

In addition to those, the risk assessment of the CQAs against the process parameters can be performed. It has well established that the process parameters voltage, flow rate and distance between the needle and collector have a very high risk towards the responses. However, these will be tested to understand the relationship between them and the CQAs. The operation and experiments preforming will be kept constant throughout the experiment, but despite this the possibility of human error will still be present, hence this has been left as medium risk.

An Ishikawa, or cause and effect, diagram is shown in Figure 6. 3, which illustrates the many factors that may potentially affect the CQAs, whether as a main cause, direct interaction with another parameter or through accumulation of experimental noise. The parameters labelled in red will be the factors to be tested and analysed to determine their relationship with the CQAs. While the other parameters will be kept as constant as possible.

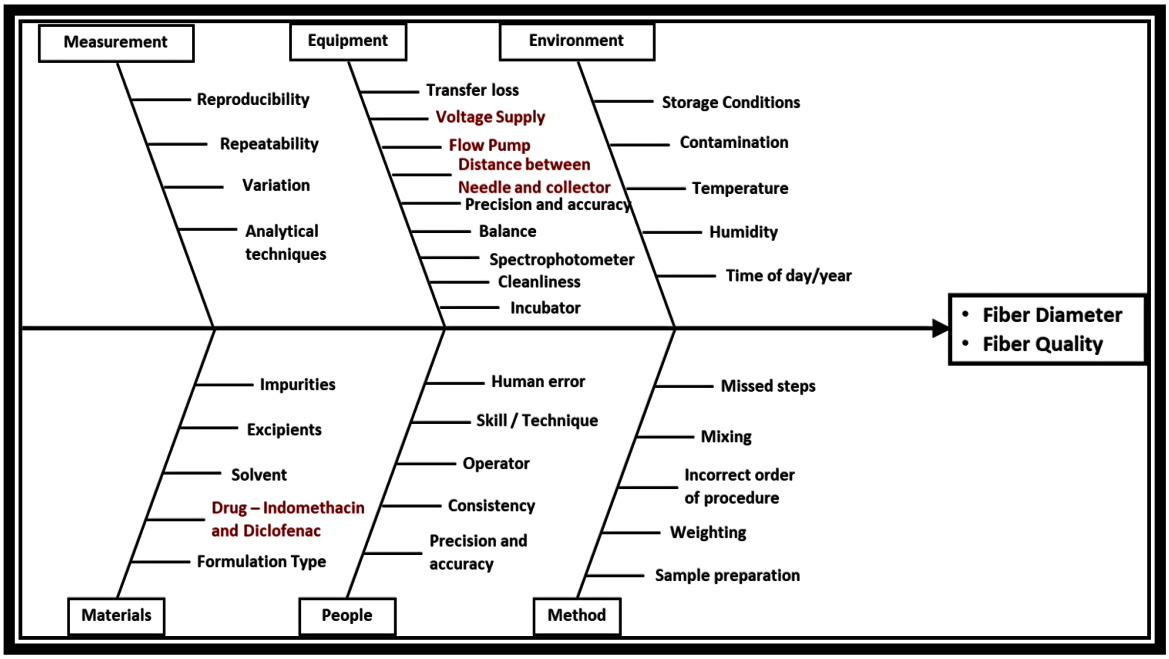


Figure 6. 3 Ishikawa diagram showing the possible factors that may affect the responses

Failure Mode and Effects Analysis (FMEA) is also a form of risk assessment that quantitatively analyses and evaluates the level of risk of CQAs being affected by material attributes and process parameters (Vohra et al., 2017). Table 6.4 shows the FMEA risk assessment for the experiment. The sources of risk are listed and their failure and effects of CQAs or responses are described in the table. Each risk was assigned a number between 1 – 5 for the three components of risk calculation: Severity of the risk (S), Probability of the risk occurring (P) and Detectability of the risk (D), where 1 represents a low risk and 5 would be a high risk. The Risk Priority Number (RPN) is the multiplication of S, P and D to give a numerical value describing the risk, which was also categorised by colour: green = low risk, amber = medium risk and red = high risk (Table 6. 4).

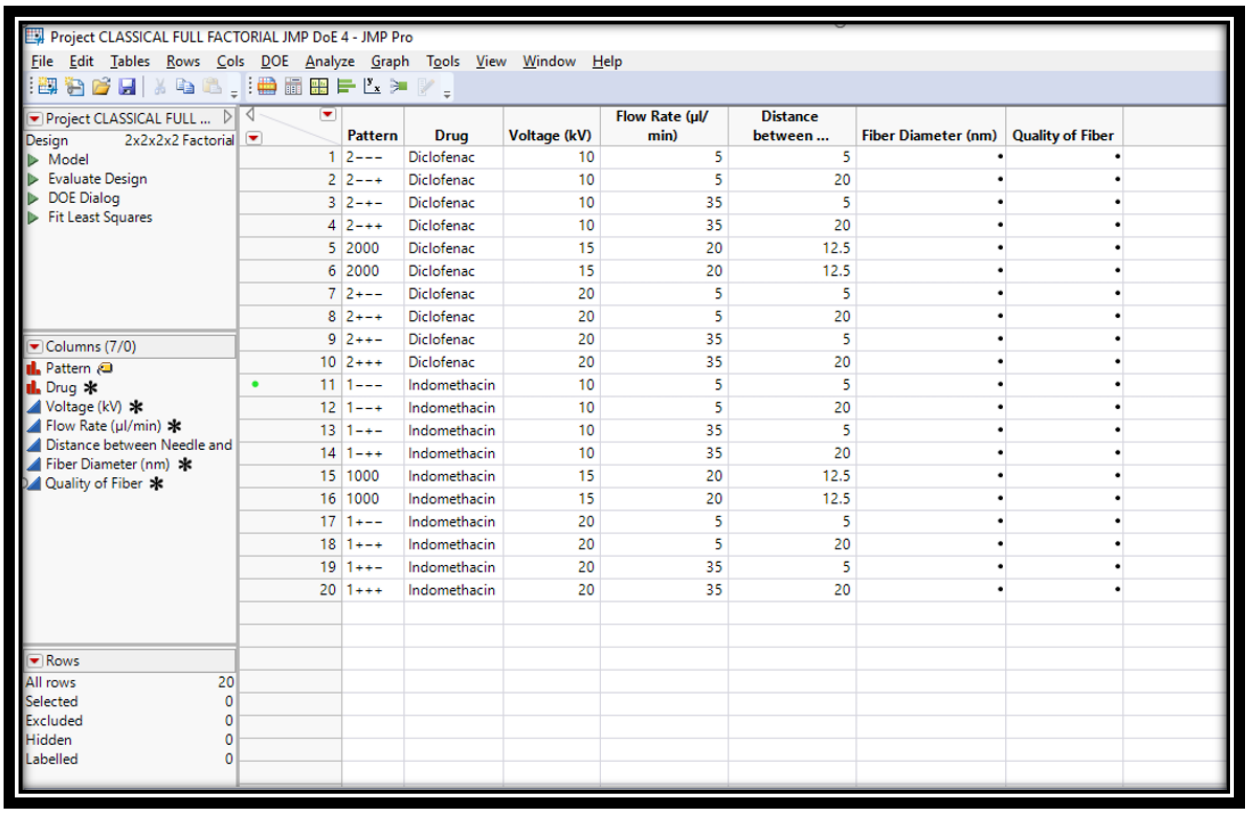
Table 6. 4 FMEA Risk Assessment

CMAs, CPPs and other sources	Failure Mode	Effect on CQAs and/or responses	Well Controlled?	S	P	D	RPN
Selected NSAID	Different NSAID may have different properties	Different drugs possess different properties – may lead to different fibre diameters and quality	No – To be tested	4	3	1	12
Voltage	Voltage too high or too low	Low – not enough to generate desired electrostatic interaction for good electrospinning technique High – too much to maintain ideal electrostatic interaction with uncontrolled whipping process	No – To be tested	5	3	2	30
Flow Rate	Flow Rate too high or too low	Low – Solution ejected too slow to make fibre desirably High – solution ejected too fast for sufficient whipping	No – To be tested	5	5	2	50
Distance between needle and collector	Distance too high or too low	Low – not enough time to sufficiently elongate and dry fibre High – Fibre may be too brittle and product spread over wider area	No – To be tested	4	3	2	24
Drug Concentration	Too high or too low	Any significant deviation between formulations will greatly affect all CQAs and invalidate results	Yes – 5% w/w and 5% w/v	5	1	1	5
Polymer Concentration	Too high or too low	Any significant deviation between formulations will greatly affect all CQAs and invalidate results	Yes – 95% w/w and 95% w/v	5	1	1	5
Polymer Molecular Weight	Too low	May not electrospin as desired, forming beaded fibres and droplets	Yes – high molecular weight PVP	4	1	1	4
Solvents Used	Different types and concentrations	Any significant deviation between formulations will greatly affect all CQAs and invalidate results	Yes – 80% v/v ethanol and 20% v/v water	5	1	1	5
Operator	Different procedures taken	More than one operator – some may make errors, others may follow different method	Yes – single operator	3	2	1	6
Density	Too high	Gravitational pull on jet may be too strong and may create fibres of poor morphology and quality	No, but easily detected	3	2	1	6
Surface Tension	Too high or too low	Surface Tension is greatly related with applied electric field in jet stream formation Too high – electrospinning may not occur Too low – uncontrolled jet stream	No, but unlikely to be high risk as using same solution	3	2	1	6
Electrical Conductivity	Too low	Too low – Poor fibre morphology with likelihood of beads forming Should be high Electroconductivity to reduce bead formation and promote cylindrical and smooth fibres	No, but easily detected	4	2	1	8
Temperature	Too high or too low	Too low – insufficient drying leading to increased diameter Too high – dries too fast and fibres may become brittle and break upon impact	No, but unlikely to change	2	1	2	4
Humidity	Too high	Causes circular pores to appear on fibre surface and may cause coalescence of fibres	No, but unlikely to change	2	2	2	8

6.6 Method of applying QbD

Prior to starting the experiment, a DoE must first be made. This will be done by using the statistical software JMP to generate a full factorial screening design. Since a total of 4 factors will be experimented, each with 2 levels, the minimum number of runs required is 16. However, centre points are also essential as they help to further understand the results by allowing the experiments to include curvature in the results. It is aimed to include 4 centre points. This brings the total number of runs to 20. By inputting the responses and factors, along with their respective limits, the JMP software constructed a design of how the parameters of the project experiments will be tested, as shown in Table 6. 5.

Table 6. 5 Full Factorial Screening design of the project experiments



	Pattern	Drug	Voltage (kV)	Flow Rate (µl/ min)	Distance between ...	Fiber Diameter (nm)	Quality of Fiber
1	2−−−	Diclofenac	10	5	5	•	•
2	2−−+	Diclofenac	10	5	20	•	•
3	2−+−	Diclofenac	10	35	5	•	•
4	2−++	Diclofenac	10	35	20	•	•
5	2000	Diclofenac	15	20	12.5	•	•
6	2000	Diclofenac	15	20	12.5	•	•
7	2+−−	Diclofenac	20	5	5	•	•
8	2+−+	Diclofenac	20	5	20	•	•
9	2++−	Diclofenac	20	35	5	•	•
10	2+++	Diclofenac	20	35	20	•	•
11	1−−−	Indomethacin	10	5	5	•	•
12	1−−+	Indomethacin	10	5	20	•	•
13	1−+−	Indomethacin	10	35	5	•	•
14	1−++	Indomethacin	10	35	20	•	•
15	1000	Indomethacin	15	20	12.5	•	•
16	1000	Indomethacin	15	20	12.5	•	•
17	1+−−	Indomethacin	20	5	5	•	•
18	1+−+	Indomethacin	20	5	20	•	•
19	1++−	Indomethacin	20	35	5	•	•
20	1+++	Indomethacin	20	35	20	•	•

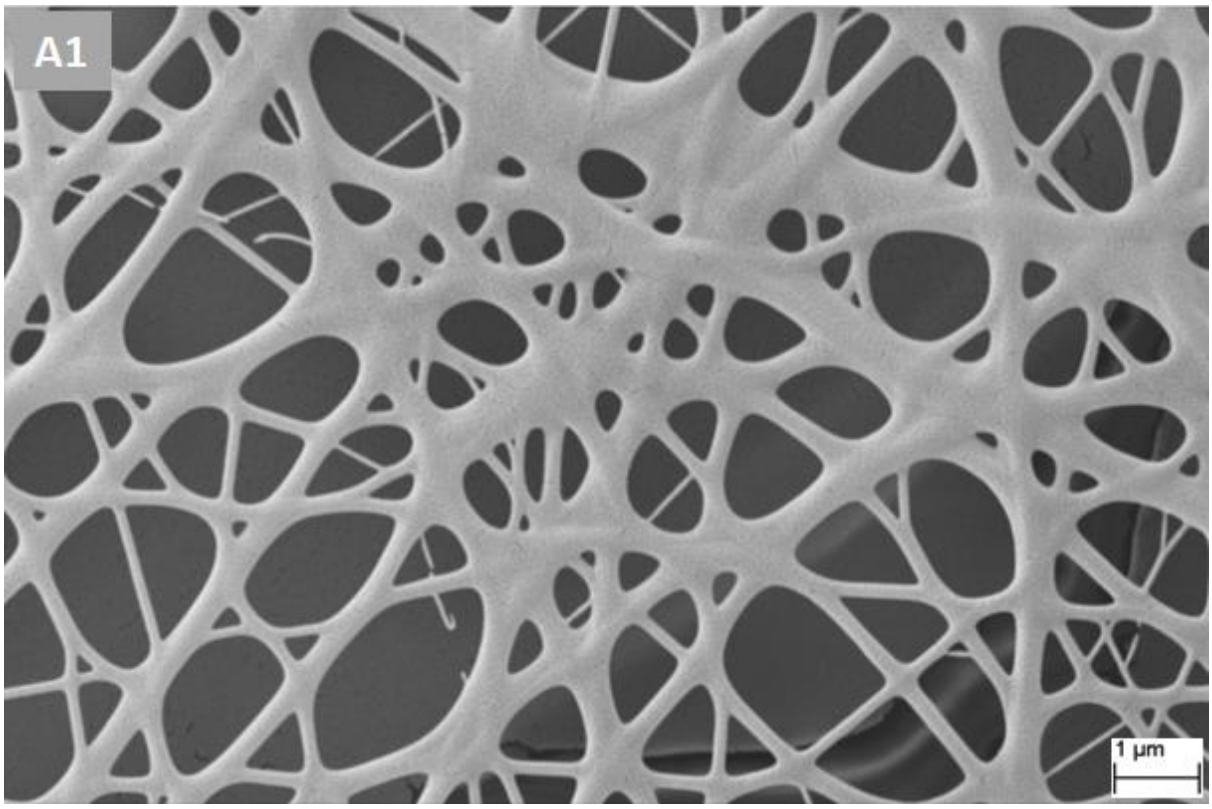
The DoE has now been established and the experiments can be performed by following this order. Before the electrospinning process can proceed, the materials must first be weighed using an analytical balance. The formulation of the fibre will follow the general formula of: 250mg of drug (indomethacin and diclofenac sodium) and 4750mg of PVP. When the correct process parameter settings have been set, the process will commence, and the pump will push the solution through the silicon tube and into the electrospinning needle, where the electric field will be applied, and the solution will be ejected on to the collector as fibres until all of the solution is ejected. This will be repeated for all 20 runs, after which testing the samples will begin.

Once all the raw data has been collected and inputted into the JMP software, the data will then be simulated and analysed. Predicted vs actual plots will be used to assess the level of deviation of the results. The ratio values can help determine the level of noise that may be incorporated in the data. Main effects and interactions of high significance can be identified. Profilers will be generated to analyse the relationship of the responses with the factors tested. This will ultimately lead to a contour plot that will help define a design space, in which working within the space will ensure that fibres produced will be of desired quality. Using this design space and the information that will be gained alongside, a control strategy can be devised to further ensure the quality of the fibres.

6.7 Results and discussion

Fibrous films were engineered using two NSAID drugs formulations (Diclofenac Sodium and Indomethacin). Stable jetting was determined using a preliminary applied voltage contrasted with infusion rate experimental matrix in accordance to Table 6. 4. Different jetting mode has been developed regard to material propertied of different drug and therefore, different structure of end product for each set up of experiments. However, stable jetting windows were determined for NSAID-PVP based formulations at center points.

Formulated fibres at center point for both NSAID drugs exhibit a smooth surface; albeit with slightly variable diameter distributions. Figure 6. 4 show surface morphology of electrospun fibrous films obtained using plan view microscopic analysis. Figure 6. 5 shows average diameter distributions of selected fibres from each electrospun membrane sample run. The fibre diameter was noted to be different for each drug for the similar run. However, both drugs show best results at center point runs. Furthermore, indomethacin shows better result for the runs after center point especially last two (high voltage and high flow rate) runs with better fibre quality compare to the same runs for Diclofenac sodium. The Diclofenac sodium formulation showed opposite results for the beginning runs (low voltage and low flow rate). Run 1 showed best quality fibre with lowest fibre diameter (low voltage, low flow rate, and lower distance). In addition, the runs after center points give not only acceptable fibre diameter but also with satisfactory quality. However, at high voltage, high flow rate, and high distance the fibre thickness is massively increased and as result the quality of fibre reduced.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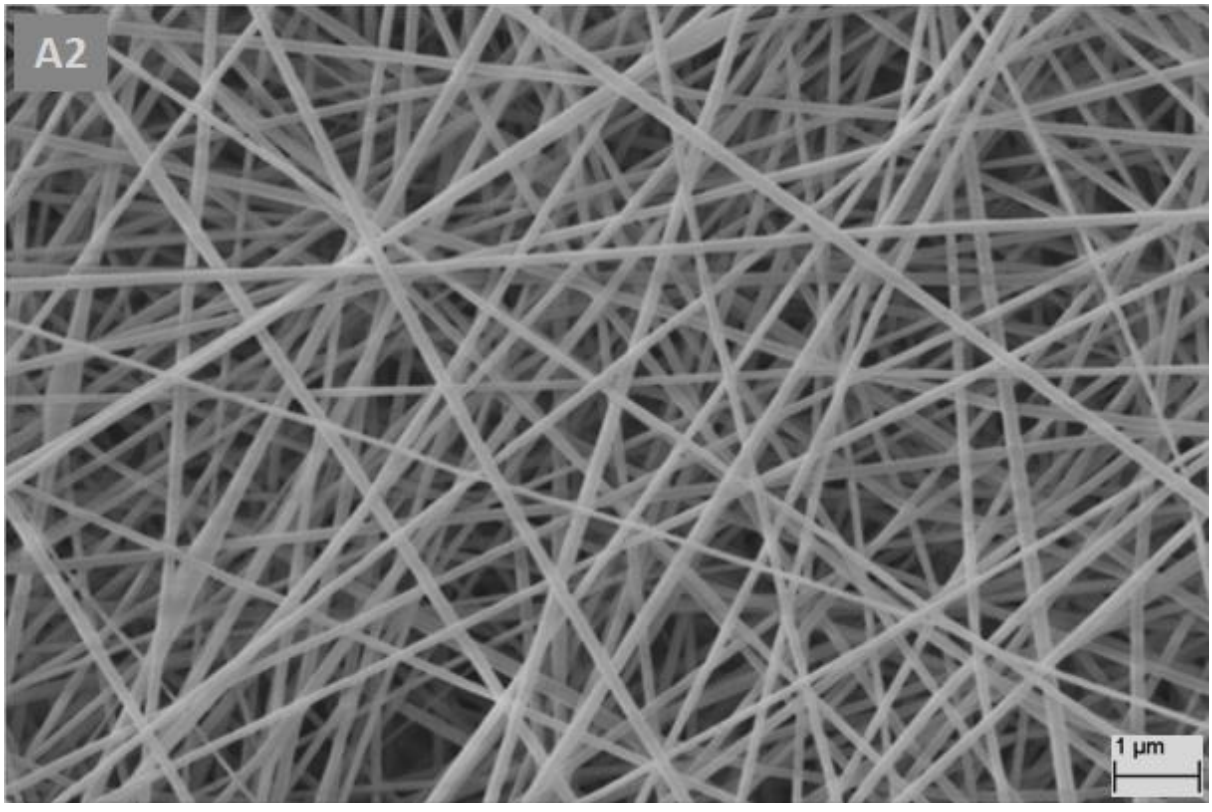
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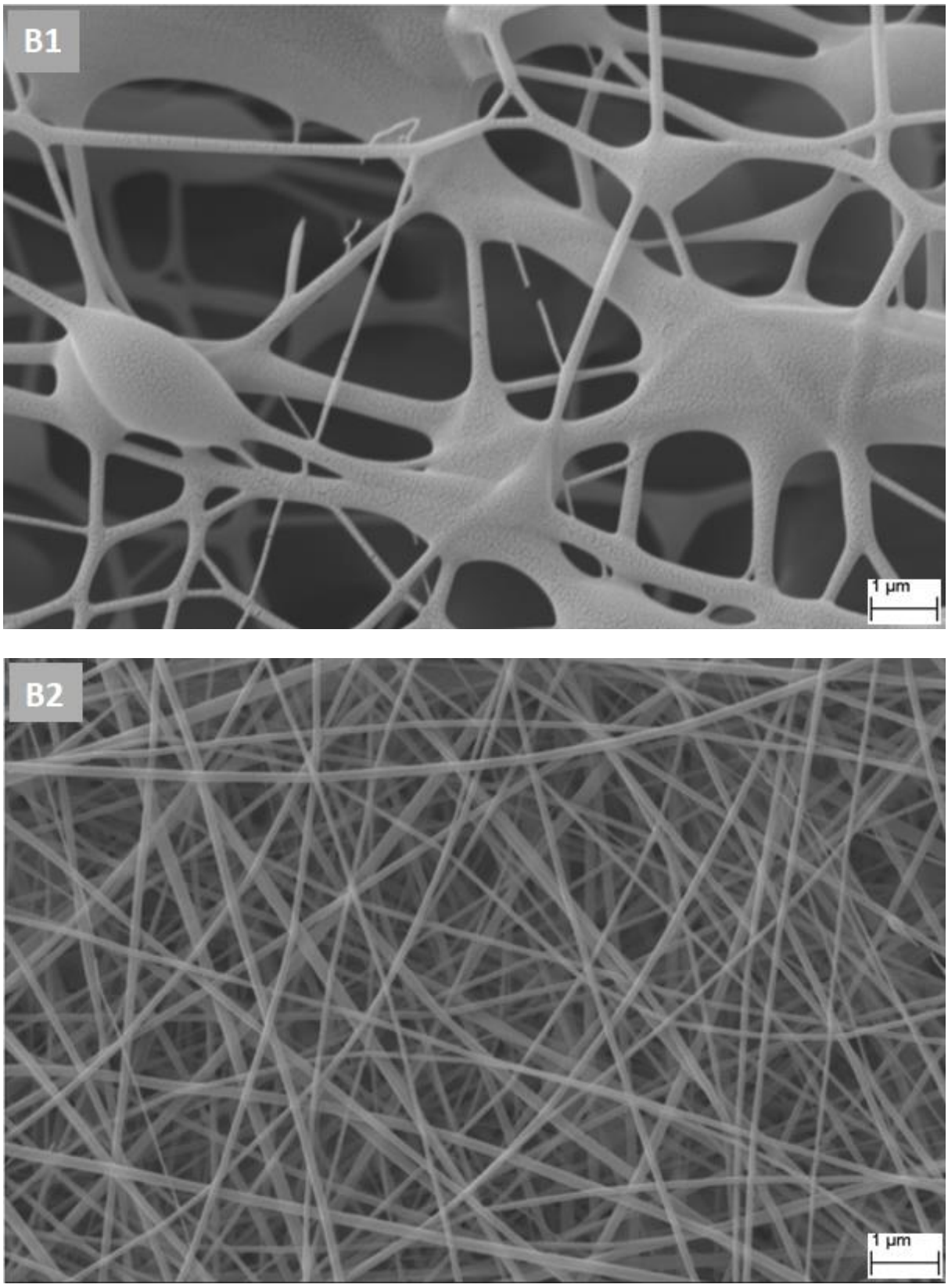


Figure 6. 4 Electron microscope of films samples of Diclofenac sodium (A1 lower quality & A2 high quality) and Indomethacin (B1 lower quality & B2 high quality) formulations. Quality of fibres identified as Low quality to high quality (1-5).

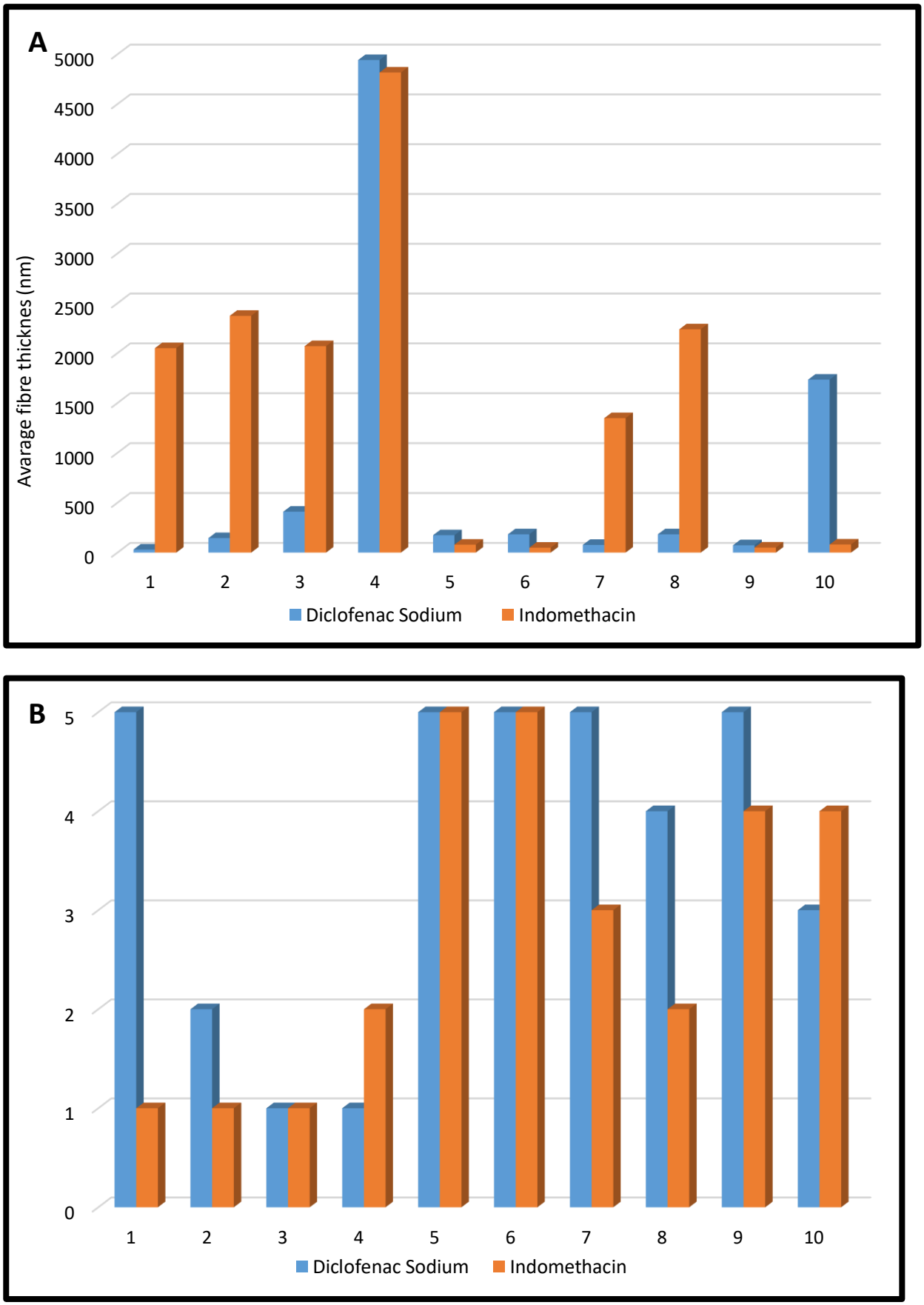
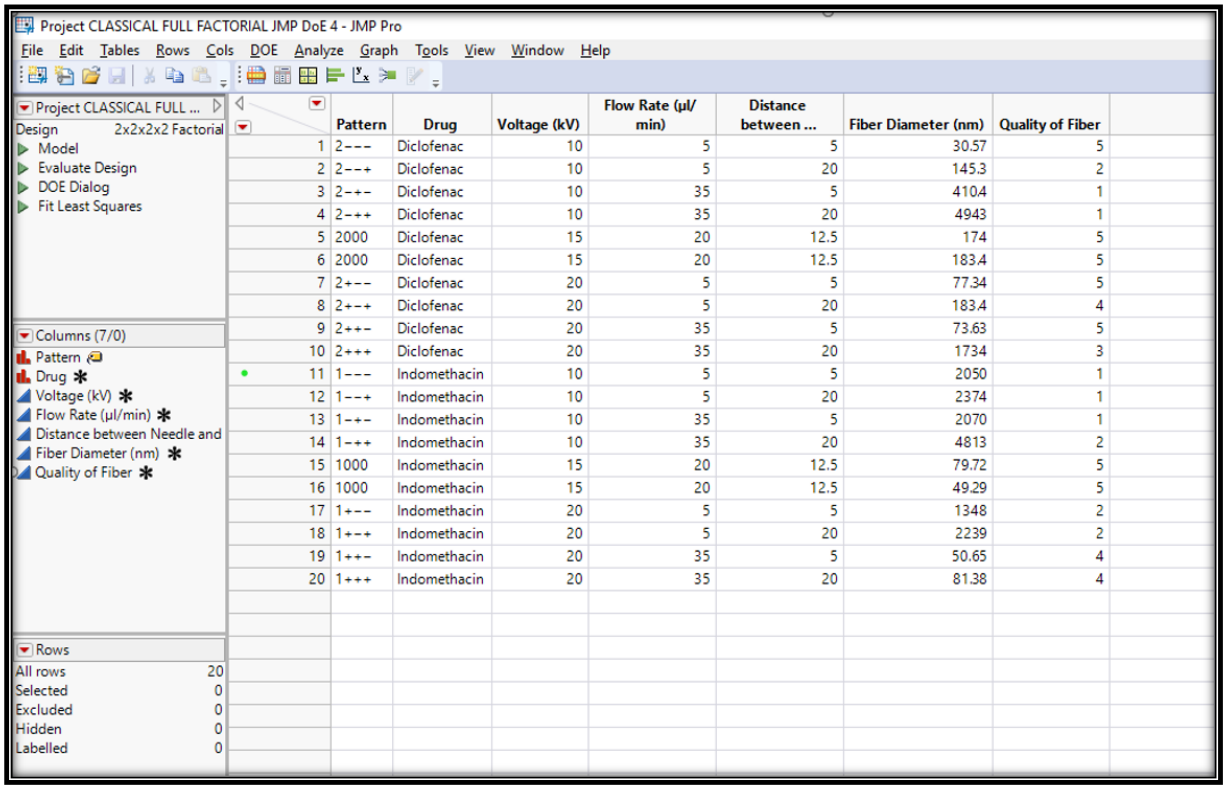


Figure 6. 5 Average fibre diameter size distribution of formulated films (A) and quality of fibres across all runs (B) (Comparing both NSAID drugs for each run).

Once all the raw data has been collected and inputted into the JMP software the data will then be simulated and analysed.

Table 6. 6 JMP table showing the results achieved from the experiments



Project CLASSICAL FULL FACTORIAL JMP DoE 4 - JMP Pro

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Project CLASSICAL FULL ...
Design 2x2x2x2 Factorial
Model
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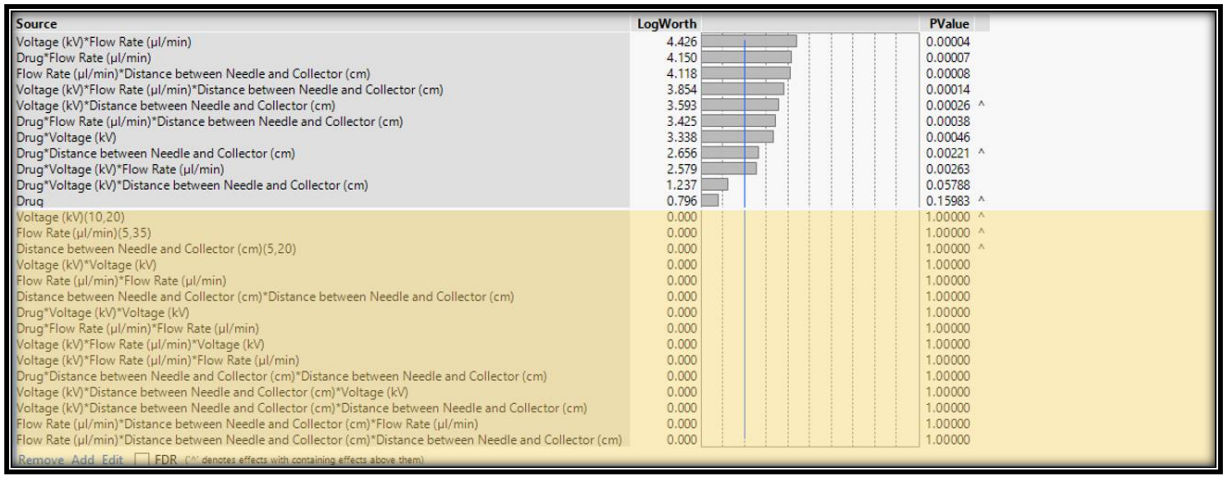
Columns (7/0)
Pattern
Drug *
Voltage (kV) *
Flow Rate (µl/min) *
Distance between Needle and
Fiber Diameter (nm) *
Quality of Fiber *

Rows
All rows 20
Selected 0
Excluded 0
Hidden 0
Labelled 0

	Pattern	Drug	Voltage (kV)	Flow Rate (µl/ min)	Distance between ...	Fiber Diameter (nm)	Quality of Fiber
1	2−−−	Diclofenac	10	5	5	30.57	5
2	2−−+	Diclofenac	10	5	20	145.3	2
3	2−+−	Diclofenac	10	35	5	410.4	1
4	2−++	Diclofenac	10	35	20	4943	1
5	2000	Diclofenac	15	20	12.5	174	5
6	2000	Diclofenac	15	20	12.5	183.4	5
7	2+−−	Diclofenac	20	5	5	77.34	5
8	2+−+	Diclofenac	20	5	20	183.4	4
9	2+−+	Diclofenac	20	35	5	73.63	5
10	2+++	Diclofenac	20	35	20	1734	3
11	1−−−	Indomethacin	10	5	5	2050	1
12	1−−+	Indomethacin	10	5	20	2374	1
13	1−+−	Indomethacin	10	35	5	2070	1
14	1−++	Indomethacin	10	35	20	4813	2
15	1000	Indomethacin	15	20	12.5	79.72	5
16	1000	Indomethacin	15	20	12.5	49.29	5
17	1+−−	Indomethacin	20	5	5	1348	2
18	1+−+	Indomethacin	20	5	20	2239	2
19	1+−+	Indomethacin	20	35	5	50.65	4
20	1+++	Indomethacin	20	35	20	81.38	4



Source	LogWorth	PValue
Voltage (kV)*Flow Rate (µl/min)	4.426	0.00004
Drug*Flow Rate (µl/min)	4.150	0.00007
Flow Rate (µl/min)*Distance between Needle and Collector (cm)	4.118	0.00008
Voltage (kV)*Flow Rate (µl/min)*Distance between Needle and Collector (cm)	3.854	0.00014
Voltage (kV)*Distance between Needle and Collector (cm)	3.593	0.00026 ^
Drug*Flow Rate (µl/min)*Distance between Needle and Collector (cm)	3.425	0.00038
Drug*Voltage (kV)	3.338	0.00046
Drug*Distance between Needle and Collector (cm)	2.656	0.00221 ^
Drug*Voltage (kV)*Flow Rate (µl/min)	2.579	0.00263
Drug*Voltage (kV)*Distance between Needle and Collector (cm)	1.237	0.05788
Drug	0.796	0.15983 ^
Voltage (kV)(10,20)	0.000	1.00000 ^
Flow Rate (µl/min)(5,35)	0.000	1.00000 ^
Distance between Needle and Collector (cm)(5,20)	0.000	1.00000 ^
Voltage (kV)*Voltage (kV)	0.000	1.00000
Flow Rate (µl/min)*Flow Rate (µl/min)	0.000	1.00000
Distance between Needle and Collector (cm)*Distance between Needle and Collector (cm)	0.000	1.00000
Drug*Voltage (kV)*Voltage (kV)	0.000	1.00000
Drug*Flow Rate (µl/min)*Flow Rate (µl/min)	0.000	1.00000
Voltage (kV)*Flow Rate (µl/min)*Voltage (kV)	0.000	1.00000
Voltage (kV)*Flow Rate (µl/min)*Flow Rate (µl/min)	0.000	1.00000
Drug*Distance between Needle and Collector (cm)*Distance between Needle and Collector (cm)	0.000	1.00000
Voltage (kV)*Distance between Needle and Collector (cm)*Voltage (kV)	0.000	1.00000
Voltage (kV)*Distance between Needle and Collector (cm)*Distance between Needle and Collector (cm)	0.000	1.00000
Flow Rate (µl/min)*Distance between Needle and Collector (cm)*Flow Rate (µl/min)	0.000	1.00000
Flow Rate (µl/min)*Distance between Needle and Collector (cm)*Distance between Needle and Collector (cm)	0.000	1.00000

Remove Add Edit FDR (^ denotes effects with containing effects above them)

Figure 6. 6 Effect summery of most significant effects and interaction

Predicted versus actual plots will be used to assess the level of deviation of the results. F ratio values can help determine the level of noise that may be incorporated in the data (any value close to one is not acceptable). Where, the lower the values, the greater the level of noise incorporated into the data. Here the F ratio is reasonably high (42.5927), which confirms the assumption that not much noise is present. Furthermore, the 'RSquare Adj' Figure 6. 7 illustrates how much data from the results was run through the simulation. The red box states that over 99% of the data was taken into account. This makes it a very reliable model.

Main effects and interactions of high significance can be identified using the p values. Those of a P value that were greater than 0.1 have been ignored. This was because all possible effects must be analysed and only the most significant effects and interactions contain a p value lower than 0.1. However, the drug with P value of 0.159 has been taken in account as a secondary interaction, since its interaction with other factors (e.g. drug*flow rate (µl/min), drug*voltage (KV), and drug*voltage (KV)*flow rate (µl/min)) are great.

The factor interactions Voltage(KV)*Flow rate (µl/min), Drug*Flow rate (µl/min), Flow rate (µl/min)*distance between needle and collector (cm), Voltage(KV)*Flow rate (µl/min))*distance between needle and collector (cm), Voltage(KV)*distance between needle and collector (cm), Drug*Flow rate (µl/min))*distance between needle, and Drug*Voltage(KV) were the most significant respectively, and interactions between Drug*distance between needle (cm), Drug*Voltage(KV)*Flow rate (µl/min), and Drug*Voltage(KV)* distance between needle(cm) were also considered highly significant Figure 6. 6. Although drug P value is slightly above the 0.1 and is not considered as highly significant but still shows some effect and considered in this simulation.

Profilers will be generated to analyse the relationship of the responses with the factors tested. This will ultimately lead to a contour plot that will help define a design space, in which working within the space will ensure that fibres produced will be of desired quality. Using this design space and the information that will be gained alongside, a control strategy can be devised to further ensure the quality of the fibres.

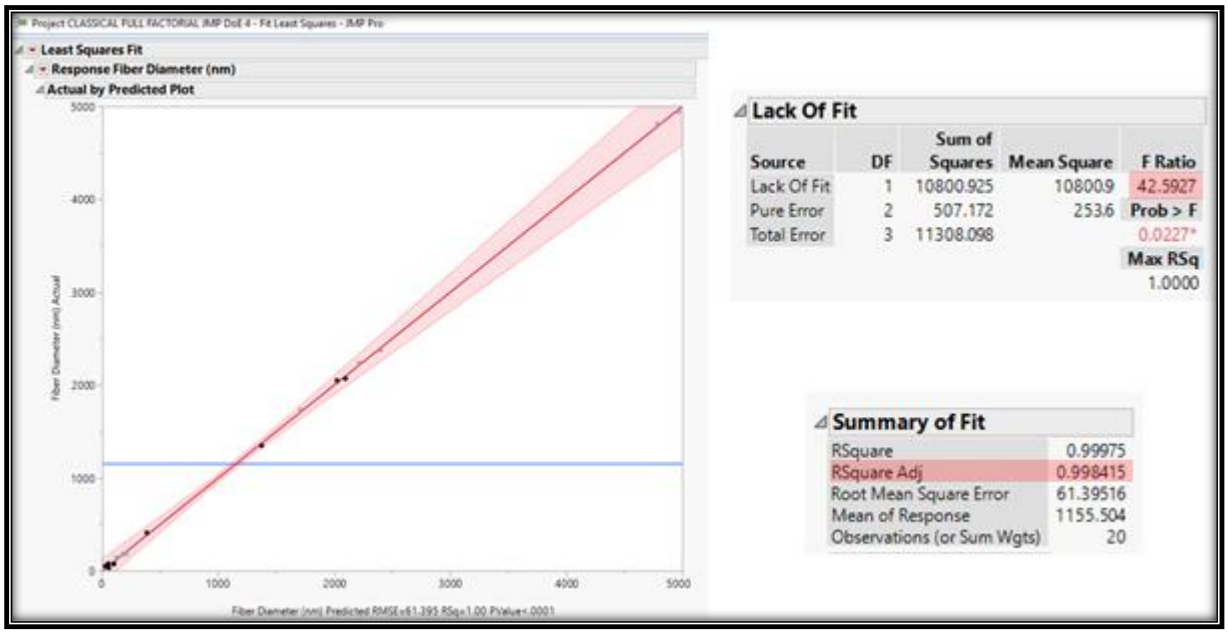


Figure 6. 7 Effect summery of fibre diameter (Actual by predicted plot for average fibre diameter)

Figure 6. 7 shows an Actual by Predicted Plot of fibre diameter in the red rectangle, which depicts the extent of deviation of the actual results (black dots) from the predicted result space (red line and red shaded area). Here the deviation is very low, where, all actual results falling within the predicted result space. This means the set of results obtained is highly accurate, the process is very robust, and the variance of the error was constant. This is further backed by the 'RSq' value of 0.99 under the Plot.

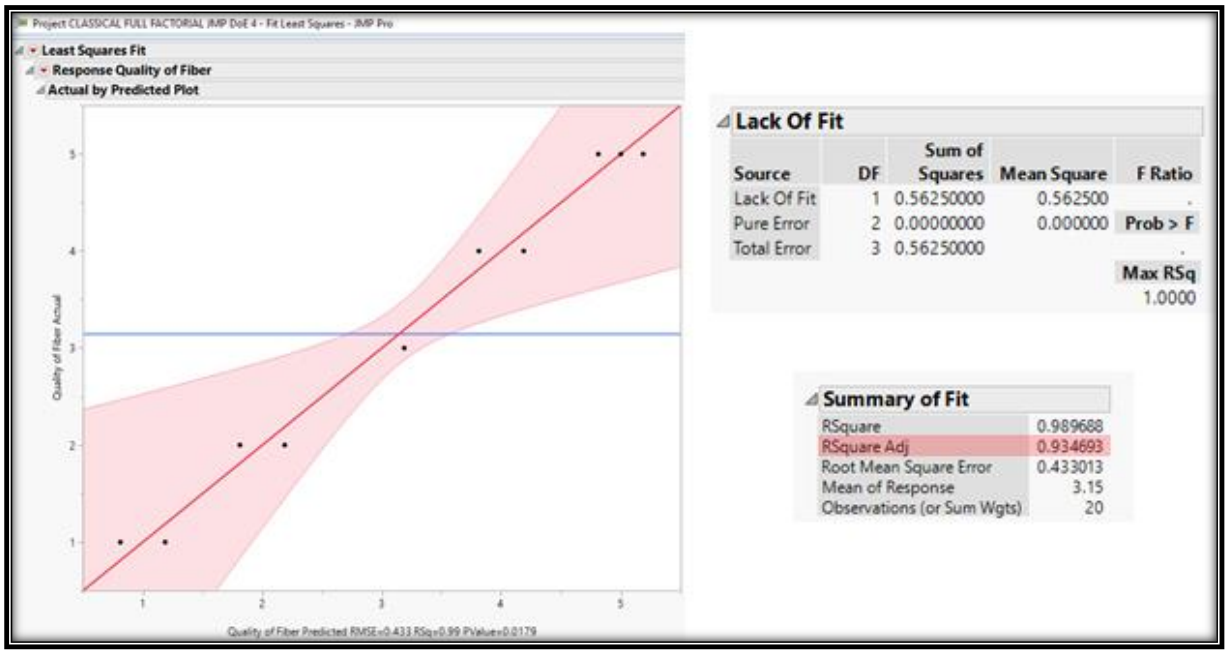


Figure 6. 8 Effect summery of fibre quality (Actual by predicted plot for average quality of fibre)

The spread of data in the results obtained for average quality of fibre was satisfactory. This can be seen by the red shaded area, where all actual results fall into predicted area. Although there is a fair amount of variation in the quality of the fibre, however they were all still within limits. This is further backed by the 'RSq' value of 0.93 under the Plot. This variation may be due to different operation electrospinning equipment or environment that fibres have been produced. However, Figure 6. 8 states that over 93% of the data was taken into account. This makes it a very reliable model.

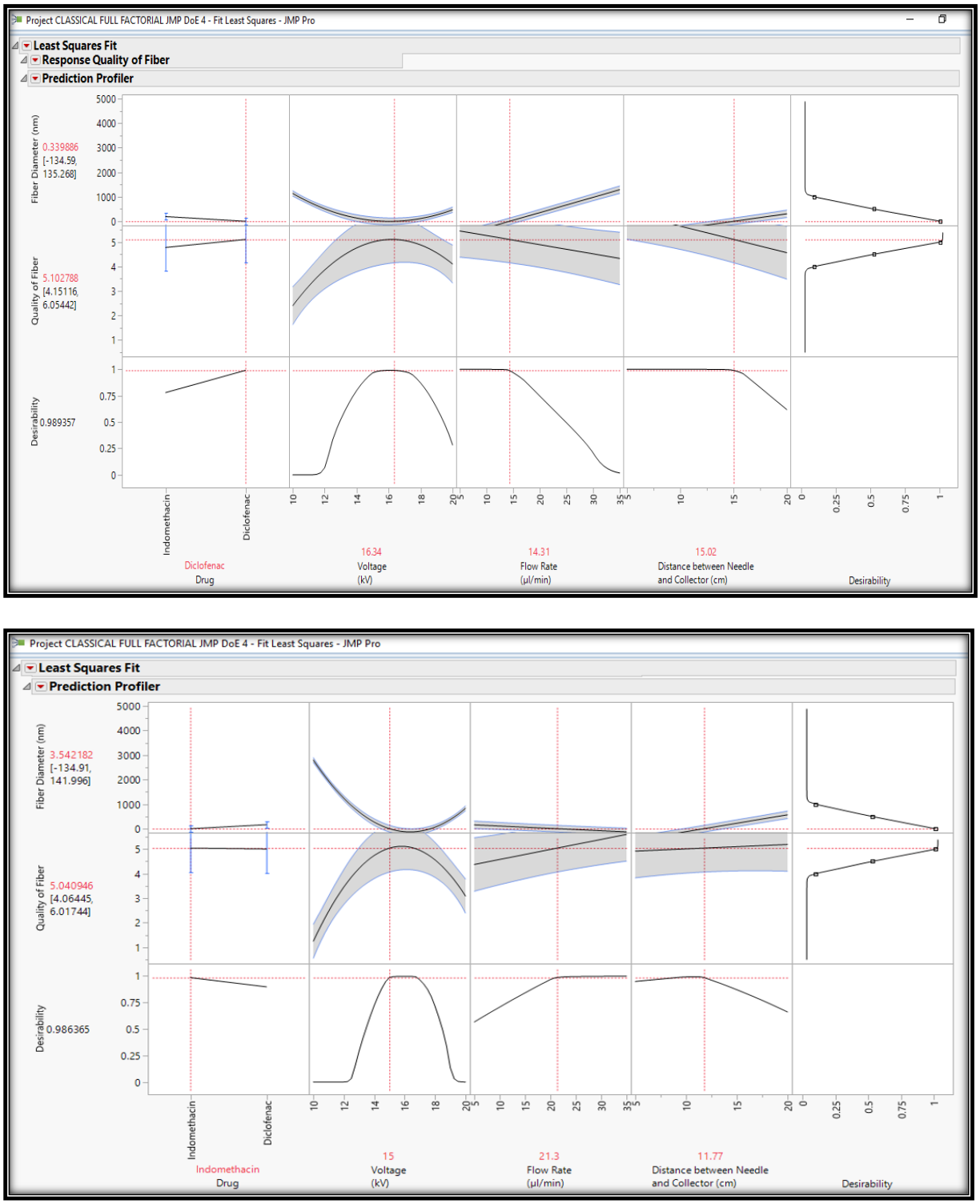


Figure 6. 9 Profilers showing the optimum conditions for each drug. (Effect of voltage, flow rate, and distance of needle from collector against desirability, quality of fibre, and fibre diameter (grey area is standard error).

Figure 6. 9 illustrates the relationship between the factors and how they affect the desirability and response of the experiment. At avarage (centre point) voltage, high flow rate, and lower distanse between needle and collector, the desirability is high for Diclofenac sodium, with average fibre quality and diameter likely to be within limits. However, increasing the distance between needle and collector will reduce the desirability, while reduce fibre quality and also increasing fibre diameter. The same events occur with high flow rate, where desirability also decreases. Flow rate greatly effect fibre quality and fibre diameter.

The desirability and response of experiment was slightly different for indomethacin. Although operation voltage is almost same for both drug for high desirability, but indomethacin had higher desirability with higher flow rate, where fibre quality and diameter likely to be within limits. However, reducing the voltage will signefectly reduce the desirability, while reduce fibre quality and also increasing fibre diameter. In jeneral,

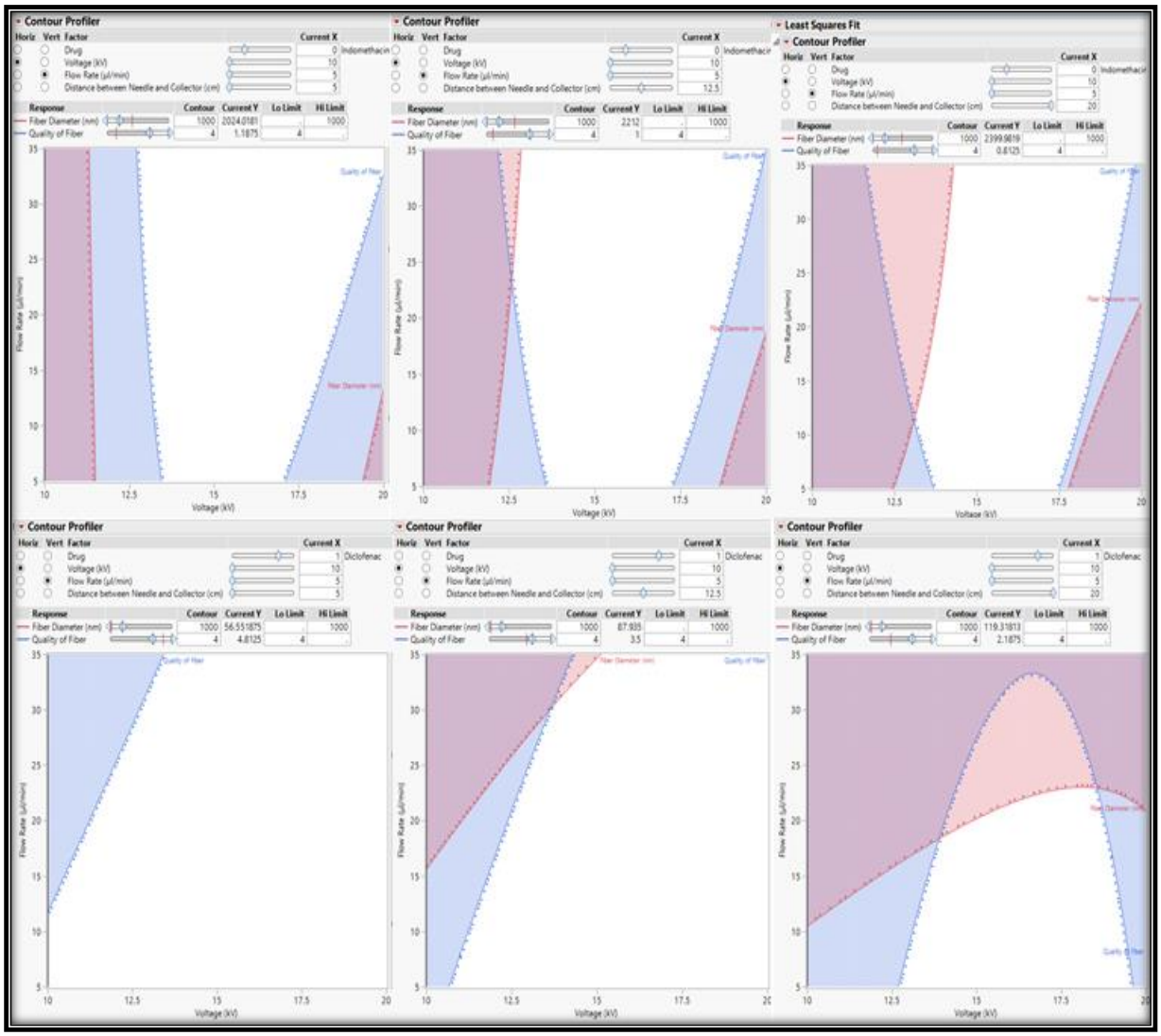


Figure 6. 10 Design spaces with Flow Rate against Voltage; (top row) Indomethacin, (bottom row) Diclofenac Sodium, (left column) at 5cm distance, (middle column) 12.5cm and (right column) 20cm

Figure 6. 10 shows the predicted contour plot of the factors flow rate and voltage, with the limits of the responses already included. This helps define the design space of the process, where working within this multidimensional space ensures that the end product will be made to the desired and required qualities.

In the image shown, fibre diameter (red) and quality of fibre (blue) affect the design space within the ranges used for the factors that were assessed. Contour plot helps to get an idea of the design space, of which the product will achieve its target if working within the limits of the design space (white area). If the operation kept in the white area the product desirability is certain. Formulation with diclofenac sodium shows most desirable conditions and opens the design space into a wider area compare to formulation with indomethacin. However, a common design space can be achieved from both contour plot which can predict high desirability for both formulations.

Through the use of a risk-based approach from the foundations of product and process understanding, a Control Strategy was created, as shown in Table 6. 7. It describes many aspects of the process that the operator can control to ensure the process is always within the acceptable limits of the CQAs. This helps to further maintain the process within the Design Space in order to secure the deliverance of electrospun fibres to the desired quality consistently.

Table 6. 7 Control Strategy for the Electrospinning of fibres

Control Strategy Element	Included (Y/N)	Rationale
Input Material Controls	Y	Use only the same materials with the same concentrations. Replacing one, adding a new material or altering the concentrations will affect the CMAs, thereby affecting the CQAs and responses, and so would require characterisation tests to be done and a repeat of the experiment to obtain better understanding to form fibres of assured quality
Procedural Controls	Y	Ensure each run is performed for the same length of time and method is followed consistently within chosen Design Space.
Process Parameter Controls	Y	The parameters chosen must be within the chosen Design Space to ensure product quality. Ensure Distance of 5cm is used if both INDO and DICLO Fibres are to be produced with the same parameter settings
In-Process Tests	N	Not required as electrospinning is a one-step technique
Specification	Y	Should meet QTPP requirements and within Design Space limits for Fibre Diameter and Quality of Fibre to promote surface area, bioavailability, stability, porosity etc.
Process Monitoring	Y	Observe process to ensure solution is being ejected with proper spray pattern and avoid forming droplets, dried fibres at needle tip and any blockages within apparatus
Characterisation and Comparability	Y	Formulation and fibres must be thoroughly characterised to gain sufficient information on its properties, especially Fibre Diameter. Fibre products must be compared and analysed between runs via SEM analysis to estimate the Quality of Fibre

6.8 Conclusion

QbD is the heritage of the experience achieved from manufacturing industries even though considered as new drug development. QbD allows the measurement and assessment of different parameters and their effects on critical properties of the methodology and furthermore support the importance of scientific knowledge to improve and optimise methodologies.

The study was performed to find the best factorial combination to get the best possible fibre quality with desire diameter when producing NSAIDs drug for buccal delivery and how robust the manufacturing process is by using QbD. This was found to be This was found to be if the flow rate stays within the range of 5-15 (µl/min), and voltage stay between 13.5 -17.5 (KV), the best quality fibre with desirable dimeter will be achieved. As a result, the process is a robust and reliable. Although these experiments allowed some understanding of the formulation and the process effects on the quality of the final product, further experiments and analysis with new drugs are needed to be carried out in order, to obtain better results and design space.

All design spaces can be used with the respective drug and parameters separately, while the design space at 12.5cm would be most suitable if the same parameter settings were to be used for both drugs. In future, verification studies could be done, along with a complete control strategy. A wider range of factors, such as more drug levels and multiple polymers, could also be studied to obtain better process and product understanding.

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Chapter 7 General conclusion and future prospective

7.1 General conclusion

The overall results suggest that EHDA technique has potential for development of NSAID formulations for buccal delivery system. However, suitable polymeric matrix to function as a degradable and stable drug carrier is needed. A selection of co-polymers in conjunction with NSAID drugs and PVP (main polymer) were analysed.

The effect of co-polymers on the encapsulation and release was processed. Although each co-polymer shows an effect on the EHDA system and electrospinning of the formulation, therefore, for each formulation the EHDA system needed to be optimised. Furthermore, the resulting electrospun were characterised using various techniques. The NSAIDs electrospun were found to be made up of fibres with diameter in range of nanometre size. The results also show that various formulation of buccal patches containing NSAID drugs can be easily engineered by mean of ES method into the amorphous state.

Furthermore, the X-ray studies demonstrate that NSAID drugs which was associated with the fibre was in an amorphous state and therefore molecularly dispersed within the matrix. As can be compared with the cast technique which is the NSAID drugs show the crystallinity from their X-ray studies.

The in vitro release of NSAID drugs was regulated by the hydrophilicity/hydrophobicity of co-polymers in the formulations. This study provides a method to design novel NSAID-polymer hybrid electrospun mats exploited for buccal delivery.

EHDA system parameters and their effect of critical properties of the methodology was assessed and measured by QbD therefore gain best possible fibre quality with desire diameter NSAID formulations. This was found to be if the flow rate stays within the range of 5-15 (µl/min), and voltage stay between 13.5 -17.5 (KV), the best quality fibre with desirable dimeter will be achieved. As a result, the process is a robust and reliable.

7.2 Future work

In this study, it has been shown that EHDA can provide a reliable fibre film for the purpose of encapsulation of the NSAID drugs for buccal application. The concept of EHDA has been highlighted in this work, however, future work required necessary to highlight the potential of buccal film produced for buccal application. The impact of produced film on cell culturing will be core investigation of future study.

Limited polymer and co-polymer were assessed during this study. There are many natural and sensitive polymers which were used in buccal (e.g. tablets, patch, gel) formulation. Characterisation of more range of polymer may demonstrate more positive results. It is possible to coaxial the drug with extra polymer to capsulate the main formulation in case slow release required (prolonged the release by extra coating)

It might be ideal to perform experiments with same formulation but with different technique to investigate the variation and drug encapsulation. Hence analysis of the end point product can provide more in-depth information about each technique and as a result validate the EHDA. This instrument is new and need to be investigated further with different formulation.

As during this work all the aspect of EDHA kept constant, it is ideal to investigate more in depth the parameters which has high effect on the final product (voltage, flow rate, and distance to collector).